

THE PRIMARY TOXICITY OF NORMAL SERUM

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ANAPHYLATOXIN AND ANAPHYLAXIS

VIII. THE PRIMARY TOXICITY OF NORMAL SERUM

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THE TOXICITY OF NORMAL RAT SERUM

SUMMARY

One finds it to be the impression of most workers who have concerned themselves with the study of anaphylatoxin, that the serum of the guinea-pig is harmless when introduced into the circulation of an

animal of the same species. From all sides, evidence is presented that the serum of the guinea-pig, in itself harmless, becomes toxic after appropriate treatment.

Stühmer,¹ while noting the work of other writers on the toxicity of homologous serum for rabbit and guinea-pig, thought that this toxic condition persists for but a short time after drawing of the blood. Several workers, among whom is Haren,² believed that normal guinea-pig serum is innocuous for like animals, as does also Tchernoroutzky.³ The latter gives from 2 to 3 c.c. per 100 gm. as the fatal dose of rabbit serum anaphylatoxin for guinea-pigs, although smaller amounts of normal rabbit serum are frequently lethal for these animals.

It would seem, then, that a study of the normal sera, unchanged by the action of alien substances, would be of considerable value. We wish to emphasize, in the beginning, that we are aware that this conception of nontoxicity is not prevalent in regard to all heterologous sera. Mosso long ago described the toxicity of eel serum. Doerr and Raubitschek,⁴ state that the serum of the eel is fatal for the guinea-pig in doses of 0.01 c.c. What is more, dog serum is toxic for the guinea-pig in doses of from 1.5 to 2 c.c. Beef, sheep, and goat sera have been shown to be fatal in somewhat similar amounts, while normal, human serum killed small guinea-pigs in doses of from 0.5 to 0.75 c.c.

Before proceeding to the experimental part of our work, it will be well to give a brief account of the various ideas of the cause of normal serum toxicity.

HISTORICAL SUMMARY

The earliest record of the toxicity of normal blood is to be found in the report of the commission of the Royal Philosophical Society, published in 1666. The commission, appointed to inquire into the cause of transfusion accidents, concluded that these were due to the intravascular clotting of the transfused blood. The early transfusion work was done usually with heterologous blood. Other explanations, besides that of thrombosis, were brought forward. Magnani,⁵ who injected sheep blood into dogs, concluded that the fatal result was due to the use of a too great volume. Bischoff⁶ thought that the venosity of the blood was the cause of its toxicity, and Brown-Sequard⁷ held a like opinion.

The work of Landois⁸ must be looked upon as opening a new era in the investigation of normal blood toxicity. He was the first to employ serum,

¹ Deutsch. med. Wchnschr., 1912, 38, p. 2123.

² Ztschr. f. Immunitätsf., 1913, 20, p. 673.

³ Compt. rend. Soc. de biol., 1913, 74, p. 1213.

⁴ Berl. klin. Wchnschr., 1908, 45, p. 1525.

⁵ Paul Scheel: Die Transfusion des Blutes, usw. Quoted by Coca.

⁶ Müller's Archiv, 1835, p. 347; Schmidt's Jahrb., 1836, 12, p. 16.

⁷ Leçons sur les phénomènes physiques de la vie, 1842, t. 4.

⁸ Die Transfusion des Blutes, Leipzig, 1875.

rather than whole blood, thus eliminating one complicating factor, the foreign, red blood cells. He used rabbits and dogs as recipients, and tested the effect of a large number of heterologous sera on these animals. Experiments in the test tube were made for the first time by Landois who found that the sera which were toxic for the rabbit also dissolved or flocked the erythrocytes. From these experiments, it was concluded that the agglutinating sera, when injected into the veins of the rabbit, cause intravascular flocking of the recipient's red blood cells and consequently capillary blocking, resulting in death. The hemolysing sera, on the other hand, were believed to kill by liberating thromboplastic substances from the erythrocytes, with resulting intravascular clotting, plugging of the lung and brain capillaries, and death.

Until quite recently, all explanations have confined themselves to the ideas of thrombosis, of hemagglutination, or of hemolysis. Capillary plugging, resulting from small clots or from flocked erythrocytes, is by far the most common explanation of the toxicity of normal serum. We shall glance at the evidence for and against these theories, considering, first, thrombosis.

Köhler⁹ injected a rabbit intravenously, with its own blood previously defibrinated, and produced acute symptoms and death. He found thrombi in the heart and large vessels, at autopsy, and concluded that the injected blood was toxic because of its high fibrin-ferment content. Schenk¹⁰ and Moldovan¹¹ also think that the toxicity of homologous, as well as of heterologous blood, is due to its thromboplastic activity. We shall have occasion to discuss the work of Moldovan in another connection.

Boggs¹² has shown that the thromboplastic activity of a fresh serum for plasma in the test tube does not run parallel to its action when introduced into the body. By the addition of a little alkali to fresh rabbit serum, he was able greatly to increase its thromboplastic action. Such serum, however, injected into a rabbit, did not change the coagulation time of its blood. Morawitz¹³ also opposed the idea of fibrin-ferment as being the cause of transfusion accidents. He gives no protocols and merely quotes v. Jürgensen's¹⁴ work. This author found that clotting occurred only when the injected blood was so fresh that it was still warm.

Loeb, Strickler, and Tuttle¹⁵ refer the toxicity of dog blood for rabbits to its ability to hemolyse the red cells of this animal. The hemolysis that occurs when the dog-serum is injected is believed to liberate clot-accelerating substances. These clots lodge in the lung capillaries and cause death. This theory is based on the fact that hirudin protects against this serum. But it is to be noted that this protection is only partial; that when a dose twice as large as one that would kill without hirudin was given, the injected animal would succumb, but without any visible intravascular clotting. This weakens the hypothesis of these investigators. Furthermore, Loeb, Strickler, and Tuttle admit that the thrombi in the heart and pulmonary vessels are essentially agonal ones; that they occur only as the animal is dying. The authors state that the capillary clots may occur before this. This assertion awaits demonstration. In the light of the present evidence, there is as much possibility of

⁹ Inaug. Diss., Dorpat, 1877.

¹⁰ München. med. Wchnschr., 1910, 57, p. 903.

¹¹ Deutsch. med. Wchnschr., 1910, 36, p. 2422.

¹² Deutsch. Arch. f. klin. Med., 1904, 79, p. 539.

¹³ München. med. Wchnschr., 1907, 54, p. 767.

¹⁴ Von Ziemssen's Handbuch d. Allgemein. Therapie, 1883, 1, p. 240.

¹⁵ Virchows Arch. f. path. Anat., 1910, 201, p. 5.

thrombosis being a phenomenon, concomitant with the intoxication, as of its being a cause of the latter.

Landois⁸ was the first to think of the hemagglutinating power of a serum as being a cause of its toxicity. Battelli¹⁶ has brought forward some interesting work in this connection. The washed erythrocytes of various species were injected into rabbits, which stood well the injection of dog, cat, and beef, red cells, but succumbed to those of the pig, sheep, and rat. Tests, *in vitro*, showed that rabbit serum hemolysed the erythrocytes of the latter, while it left intact those of the former group of animals. In another paper, Battelli¹⁷ showed that, while beef and dog red blood cells are harmless to normal rabbits, they were toxic to those specifically immunized. He also showed that the accident resulting from injection was not due to products liberated during hemolysis. On the other hand, the red cell stromata might be substituted for the intact cells without altering the effect described above. While Battelli's conclusion is that the cause of the toxicity of the cells is due to their flocking, by specific hemagglutinins of the rabbit serum, it is more likely that we have here the first clean-cut demonstration of red-blood-cell anaphylaxis.

Coca¹⁸ injected washed heterologous erythrocytes into rabbits, and he, too, concludes that the resulting shock is due to an agglutination of these cells in the capillaries. He does not believe that this flocking is due to the presence of a specific agglutinin only, but comes about through the influence of some substances residing probably in the capillary walls. Loeb, Strickler and Tuttle¹⁹ think that the toxicity of beef serum for rabbits is due to its hemagglutinating properties.

The precise researches of Zinsser¹⁹ have undermined largely the validity of the hemagglutination hypothesis. This author, testing the effect of goat serum upon rabbits, showed that the red-cell-flocking properties and toxicity do not run a parallel course. Fresh goat serum flocks rabbit erythrocyte energetically. It is also very toxic for the rabbit. But while heating at 56° for 20 minutes destroys its toxicity, it leaves the agglutinating power, intact. The same relation may be shown by keeping the serum for a few days.

Of the three hypotheses mentioned above, it remains to discuss that of hemolysis. Landois' work was, also, in this instance the starting point. He showed that a serum that will dissolve red cells of a rabbit will also kill the animal. It must be emphasized here that such a demonstration does not correlate the two properties. Pfeiffer²⁰ showed that normal heterologous sera caused infiltrations when injected subcutaneously into guinea-pigs. He concluded that the effect was due to the hemolysin in the heterologous serum. Heating at 56°, according to Pfeiffer, destroyed the hemolytic, but not the toxic action. In his opinion, the hemolysin of the inactive serum was reactivated in the body of the guinea-pig by its own alexin. On the contrary, Uhlenhuth and Haendel²¹ have shown that guinea-pig alexin does not reactivate the toxicity of inactive beef serum, although it restores, to a large extent, its hemolytic power.

The last mentioned investigators studied particularly the necrotizing action of foreign sera when injected subcutaneously into guinea-pigs. While the experiment mentioned above refutes in a large measure Pfeiffer's claims,

¹⁶ Compt. rend. Soc. de biol., 1904, 56, p. 1041.

¹⁷ Compt. rend. Soc. de biol., 1904, 57, p. 17.

¹⁸ Virchows Archiv f. path. Anat., 1909, 196, p. 92.

¹⁹ Jour. Exp. Med., 1911, 14, p. 25.

²⁰ Ztschr. f. Hyg. u. Infektionskr., 1905, 51, p. 183.

²¹ Ztschr. f. Immunitätsf., 1909, 3, p. 284.

another observation does this even better. The serum of shoats and of adult pigs dissolves the erythrocytes of the guinea-pig, but only that of the adult pig causes infiltration and necrosis when injected subcutaneously.

As early as 1904, Camus and Gley²² made an important contribution to the solution of this question. These workers showed that the red blood cells of the marmot are very resistant to the action of eel serum. It requires one-twentieth to one-fiftieth dilution in physiologic salt solution to produce hemolysis in from 15 to 20 hours. Dog serum dissolves the same cells in an approximately like titer (one-twentieth to one-fortieth). But while a dose of eel serum of 0.02 c.c. per kilo kills the marmot, dog serum is tolerated by this animal in doses of from 5 to 10 c.c. per kilo. Further, while the erythrocytes of the marmot are susceptible only to high concentrations of eel serum, yet the killing dose is six times as small for the marmot as for the rabbit, whose red cells are dissolved in 1/15,000 dilution of this serum. This experiment dissociates very well the hemolytic and toxic action of eel serum. If one had here to rely only on the analogy of heat destruction, the opposite conclusion would have been reached, since heating at 56° destroys the hemolytic power and, also, to a large extent, the toxicity.

Zinsser¹⁹ found that goat serum, upon inactivation, loses both its toxic properties for the rabbit, and its power to dissolve the red cells of this animal. Guinea-pig serum (alexin) reactivates the hemolytic property but not the toxic power. Furthermore, active goat serum, which has been first treated by rabbit cells so that the sensitizers and alexin are completely removed, retains its original toxicity. The objection might be raised that this toxicity may be due to products resulting from the hemolysis occurring during this treatment. Zinsser discounted this by control injection of equivalent amounts of the products of laked rabbit erythrocytes. Such extracts were shown to be harmless. Battelli²⁰ and others have demonstrated that the products resulting from the laking of red cells are harmless, while Coca has shown that hemolysis can occur, *in vivo*, without a fatal result.

In 1910, the toxicity of normal blood was correlated for the first time with the phenomenon of anaphylaxis by Doerr.²³ According to Doerr, both the symptom complex and the autopsy findings, after normal serum injections, correspond closely to those of anaphylaxis. Doerr and Moldovan²⁴ observed while studying the effects of fresh normal beef serum on guinea-pigs, that a syndrome, identical with that of anaphylactic shock, occurs; that atropin protects; and that anti-anaphylaxis can be demonstrated by preliminary small injections.

Zinsser,¹⁹ observing the autopsy findings on rabbits which had been killed by intravenous injections of fresh goat serum, arrived at a somewhat different conclusion. Blood coagulation was delayed, but there was no Auer-Lewis phenomenon, which Doerr and Moldovan had found present in the work described above, nor could Zinsser protect by means of atropin. He also failed to protect by preliminary small injections. On the other hand, the characteristic temperature depression, blood pressure drop, and alexin disappearance did occur. It is well to remember that these workers were testing the toxic effects of foreign sera on different species and that their findings are not, therefore, strictly comparable.

²² Compt. rend. Acad. d. sc., 1905, 140, p. 1717. Arch. internat. de pharmacodynamie, 1898, 5, p. 261.

²³ Ztschr. f. Immunitätsf., R, 1910, 2, p. 117.

²⁴ Ztschr. f. Immunitätsf., 1910, 7, p. 223.

Doerr²⁵ believed that the toxicity of normal blood, especially that of homologous blood, occurs according to a mechanism similar to that which causes the toxification of a serum by the introduction of bacteria or inert substances, such as kaolin or colloidal silica. Doerr's interpretation is briefly this: Blood or blood serum contains preformed toxic substances held in abeyance by hindering bodies. The removal of these hindering bodies by some absorbant disturbs the colloidal equilibrium of the serum, with a resulting liberation of the toxic substances. Thus, when clotting occurs, the precipitating fibrin acts as the disturbing element. According to Doerr, the unmasking of the toxin during clotting is transient, since the blood rapidly becomes harmless. Doerr does not interpret Moldovan's¹¹ findings on the toxicity of the blood of rabbits for homologous animals on a basis of thromboplastic activity. Further, in investigations of which he does not give the protocols, Doerr²⁵ states that it is not necessary to defibrinate with glass pearls to make a blood toxic, but that blood which clots slowly in paraffined vessels also undergoes this temporary change. Not only the whole blood, but also the serum, and even the blood cells are toxic shortly after coagulation sets in.

Slatinéano and Ciuca²⁶ showed that fresh guinea-pig serum, injected three-quarters of an hour after the blood had been drawn, is toxic in large doses to homologous animals; that this toxicity drops off within 48 hours like that of heterologous serum; and that the toxic power lost by aging can be reactivated by the introduction of a foreign colloid, such as semi-solid agar gel (Bordet²⁷). These workers agree with Doerr in that they look upon the toxicity after clotting and the subsequent reactivation by agar as due to identical mechanisms. The work of W. H. Schultz²⁸ is suggestive in this connection. He found that freshly drawn homologous blood applied to normal, smooth muscle produced no response in such muscles until clotting set in. When this became visible, a violent contraction of the muscle resulted. This would indicate that disturbances resulting in the blood toxification do not set in until clotting occurs. Schultz apparently did not investigate the effect of aging on the power of a homologous serum to produce contractility.

Zadik,²⁹ on incubating guinea-pig serum, found that this serum in a dose of 4 c.c., at first harmless, becomes toxic for homologous animals in from 2 to 3 days, and that this toxicity disappears in from 4 to 6 days. In the majority of researches upon anaphylatoxin, the investigators state that guinea-pig serum is harmless and only becomes toxic after one incubates the serum with bacteria or with a foreign colloid. From the work referred to above, it is seen that this is not the case, and that the toxicity induced is only an intensification of a state of affairs already present in the untreated serum.

The serum of a rabbit furnishes a still better reagent for the demonstration of this relationship. Here, the normal toxicity for the guinea-pig is more intense than is that of homologous serum; further, the drop in toxicity after defibrination is neither as rapid nor as great. Moldovan¹¹ states that freshly defibrinated rabbit blood is toxic for guinea-pigs, but he does not mention in what dose it is fatal. Gräfenberg and Thies³⁰ state that the serum of pregnant rabbits is fatal to guinea-pigs in doses of about 2 c.c. per 100 gm. Immediately

²⁵ Wien. klin. Wchnschr., 1912, 25, p. 339.

²⁶ Compt. rend. Soc. de biol., 1913, 74, p. 631.

²⁷ Compt. rend. Soc. de biol., 1913, 74, p. 225.

²⁸ Physiological Studies in Anaphylaxis, Bull. Hyg. Lab., U. S. P. H. S., 1912, 80.

²⁹ Folia serol., 1911, 7, p. 865.

³⁰ Ztschr. f. Immunitätsf., 1911, 9, p. 749.

after the birth of the young, the toxicity rises to 1 c.c. per 100 gm. No mention is made of the toxicity of normal, male rabbit serum for guinea-pigs. These workers do not agree with Moldovan¹¹ and with Schenk¹⁰ that death is due to thromboplastic substances. They state that the symptom complex and the autopsy findings are identical with those of anaphylaxis. Doerr and Weinfurter²¹ found normal, rabbit serum to be fatal to guinea-pigs in doses of about 3 c.c. per 100 gm. The toxicity of rabbit serum could be increased about 3 times, by bleeding the rabbit frequently over a period of 100 days or more. It is the conclusion of Doerr and Weinfurter, too, that the symptoms and the findings, at section, resemble those of anaphylactic shock.

Mita and Ito²² tested the toxicity of serum from a large number of rabbits. Once they succeeded in obtaining a very toxic serum, the lethal dose being 0.5 c.c. per 100 gm. But in the majority of instances, the fatal dose ranged from 1.5 to 2 c.c. per 100 gm. These workers claim to show a drop in toxicity after the blood has remained for some time at room temperature. This falling off is supposed to occur in from 1½ to 4 hours after bleeding. A study of their protocols does not show this toxicity decrease in a very convincing manner. In fully one-third of the cases, no drop at all can be detected. Inactivation, according to Mita and Ito, stabilizes the toxicity of rabbit serum. These workers draw no conclusion as to the reason for this toxicity, and they do not indicate that they have performed autopsies on the animals killed.

Little stress is laid by the majority of workers on the relationship between the toxicity of heterologous and homologous blood and the method of defibrination. Moldovan¹¹ does mention such a relationship. He states that rabbit blood, which is shaken vigorously for 5 minutes with porcelain beads, is more toxic than blood defibrinated for a shorter period of time. He also makes the observation that rabbit blood is not toxic for homologous animals, when it is injected before clotting. Still earlier, Studzinski²³ mentions a failure to make a homologous blood toxic for dogs, when such blood is defibrinated with a broom-like contrivance. On the other hand, he finds that blood defibrinated with beads is very poisonous. In almost all cases, those investigating this problem are vague as to the details of the technique used in preparing the serum under test. We believe that the toxicity of normal guinea-pig and rabbit sera is largely influenced by the way in which the process of coagulation occurs, a fact which we propose to demonstrate in the experimental part which follows.

EXPERIMENTAL PART

THE TOXICITY OF NORMAL RABBIT BLOOD AND SERUM

The Toxicity of Fresh, Undefibrinated Rabbit Blood.—We propose to take up, first, the toxicity of normal rabbit whole blood and serum. Its relation to coagulation will be particularly studied in order to gain a basis for a sound interpretation of its nature and origin. It is important to know whether the circulating blood of normal animals is toxic, or whether it becomes so as a result of extravascular changes. At the outset of this study, it was assumed that a normal blood or serum, such

²¹ Zentralbl. f. Bakteriöl., I Orig., 1913, 67, p. 92.

²² Ztschr. f. Immunitätsf., 1913, 17, p. 586.

²³ Zentralbl. f. Physiol., 1909, 23, p. 755.

as that of the rabbit, possessed reasonably constant qualities; indeed, it was believed that the blood of normal animals was perforce nontoxic, since it seemed difficult to understand how an animal could be seemingly in perfect condition, and yet, a carrier of poison. As the work broadened, it was realized that different species of animals showed extreme variation as to their susceptibility to anaphylatoxin, and that even animals of the same species exhibited considerable variation. These facts must be borne in mind in connection with the observations to be presented.

In the work which follows, strictly normal rabbits were used; they were all large or medium sized. In general, they were taken from the pen in the morning, before the time of feeding. By this procedure a clear, non-lipoidal serum was invariably obtained. The recipients were usually guinea-pigs, varying in weight from 170 to 210 gm.; to a less extent, white rats were also used. The latter, as in the case of anaphylatoxin work in general, proved to be of extreme value. The criterion of death, in the guinea-pig, was the nasal reflex, which occurs in practically all cases of shock which terminate fatally.

In order to determine how soon the blood of a normal rabbit, after its withdrawal from the body, became toxic, recourse was had to transfusion experiments, such as are given in Table 96. For these tests, the blood was drawn from the heart, by direct puncture of the thoracic wall, into a syringe, previously rinsed in 0.85% salt solution. It was then kept in the syringe for a given time (column headed, "interval"), and injected into the exposed jugular vein of a guinea-pig. Each test represents a separate puncture; the time required to draw up the blood into the syringe ranged from 7 to 20 seconds, while that for the injection was from 3 to 15 seconds. One rabbit served for tests Nos. 1 to 6, inclusive; another for test No. 7, and still another for test No. 8.

It will be seen from Series A, Table 96, that in the dose employed, the rabbit blood, up to about 3 minutes after being drawn, was but slightly toxic; when kept in the syringe for 3 minutes it caused a severe reaction, while that held for $3\frac{1}{4}$ minutes produced fatal shock. The symptom complex resembled closely that of true anaphylactic shock. Autopsy performed 3 minutes after death showed the Auer-Lewis phenomenon, persistence of heart beat, and fluid blood. Very small clots were present in the right heart of Nos. 5, 6, and 7.

At the stage where the injection of 3 c.c. resulted fatally, no visible clot could be detected in the syringe. Although the needle was very

fine, the blood passed through easily during the injection. It is very likely, however, that a change in the aggregation of fibrinogen had already occurred. It is not necessary for such change to be visible to the eye. A microscopic or submicroscopic alteration in the aggregation might have accompanied the disturbance which induced the toxicity noted.

Very large amounts of undefibrinated blood can be injected without injury, provided the transfusion be sufficiently rapid. Thus, with a transfer time of 30 seconds, 10 c.c. of the blood of a rabbit were transfused into guinea-pig No. 8 with practically no ill effect. This and similar tests seemed to show that the blood of a normal rabbit

TABLE 96

TRANSFUSION OF NORMAL RABBIT BLOOD TO THE GUINEA-PIG (A); AND TO THE RAT (B)

Series	Recipient			Interval* (min.)	Total Time	Result
	No.	Weight	Blood Trans- ferred (c.c.)			
A Guinea-Pigs†	1	210	3	½	50"	Nil
	2	211	"	1	1'14"	Moderate
	3	207	"	2	2'16"	Slight
	4	180	"	3	3'21"	Severe
	5	209	"	3½	3'39"	4'56". Typical shock
	6	220	"	"	3'50"	3'17". " "
	7	173	"	"	3'23"	4' 7". " "
	8	210	10	"	30"	Slight
B Rats	1	125	3	½	1'15"	Nil
	2	120	"	1	1'40"	"
	3	130	"	2	2'39"	Slight
	4	140	"	3¼	3'57"	2'38"

* This represents the time from the end of bleeding to the start of injection, full time in syringe. Total time is the period from the start of drawing the blood to the end of the injection of guinea-pig or rat.

† See Tables 69, 97, and 98.

was nontoxic, provided it was rapidly transferred. This conclusion, however, is not strictly true, for it has been shown in connection with Table 69 that it is possible for apparently normal rabbits to have a blood which possesses a high initial toxicity. The transfusion of 2 c.c. of heart blood, even at the maximal speed of less than 20 seconds, may cause acute death in guinea-pigs. Of 21 rabbits thus tested, 4 were found to have such toxic blood; the records of 3 of these are to be found in Table 69, and that of the fourth is given in Table 97, where it will be noted that even 1 c.c. of the circulating blood, was acutely fatal though the transfer time was but 10 seconds.

The variation in the toxicity of normal rabbit blood becomes readily understood when it is recalled that this animal is very resistant to anaphylatoxin (Part V). It may tolerate a considerable number of guinea-pig lethal doses without any ill effect; hence, if through some unknown cause, 50 or 100, or even 200 of such doses are generated within the body, the rabbit may carry them without any apparent change in its general condition. The normal rabbit, therefore, is subject to great variation as to the inherent toxicity of the circulating blood, and it follows that the primary toxicity of its serum may be expected to show a like variation. The chief reason for this change in the quality of the blood is probably to be found in the food. Thus, of 2 rabbits, kept side by side for 3 months, one on an exclusive diet of cabbage, and the other on carrots, the former gave a heart blood which caused acute death in dose of 5 c.c., the transfer time being 22 seconds, whereas 3 c.c. were without effect. The rabbit fed on carrots had a less toxic blood, since 7 c.c. produced but a very slight effect, the total time of transfusion being 43 seconds; while 10 c.c., with a transfer time of 1'10", proved fatal in 6 minutes.

In Series B of Table 96 are given transfusion tests from a single rabbit to white rats. It will be observed that this species responds to pre-clot blood in the same way as the guinea-pig. In dose of 3 c.c., the rabbit's blood was without effect until it had been held in the syringe for 3 $\frac{1}{4}$ minutes when it resulted in acute death. The autopsy was typical of anaphylatoxin except for the presence of a slight clot in the heart. The symptoms in rats injected with preclot blood are of the usual anaphylatoxin type: dyspnea, spasms, convulsions and marked drop in blood pressure. It is rather striking that the rat should be about as susceptible to pre-coagulation toxicity as the guinea-pig, although, weight for weight, the rat can tolerate 100 times as much anaphylatoxin as the latter. It would seem from this as if the toxicity of the blood in the pre-coagulation stage was not due entirely to anaphylatoxin; it is necessary to conceive of a labilized state which induces the production of this poison in the blood of the recipient. That such is the case will appear from the transfusion experiments given in Table 100.

Rapid Decrease in Toxicity of Fresh Rabbit Blood.—It has just been shown that the blood of a normal rabbit may suddenly acquire toxic properties in the period immediately preceding visible coagulation. It is pertinent to inquire whether this poisonous quality persists

in the defibrinated blood after coagulation, and is maintained in the free serum. The effect of various conditions on the production and maintenance of toxicity will be considered later on, but at this point it is desirable to bring out the main fact that a decrease occurs in the toxicity of the serum as compared with that of the blood or its plasma.

It will be seen from test No. 3, Table 97, that even 1 c.c. of the heart blood of a normal rabbit, under maximal speed of transfusion of 10 seconds, was acutely fatal. On the 10% basis, the rabbit carried 221 c.c. of blood, or that number of guinea-pig lethal doses; expressed in other words, it carried per 200 gm. of body-weight, 20 of such lethal doses. In view of this high toxicity of the whole blood, it was decided to test that of its plasma, and of its serum.

TABLE 97
COMPARATIVE TOXICITY OF A NORMAL RABBIT'S HEART-BLOOD, PLASMA, AND SERUM

Donor		Recipient		Intravenous Injection (c.c.)	Total Time	Result
No.	Weight	No.	Weight			
15	2212	1	195	2 blood	16"	2'45"
		2	208	" "	15"	2'30"
		3	200	1 "	10"	3'
		4	197	0.5 "	14"	Very slight
		5	202	0.6 plasma	6'	6'30"
		6	201	1.0 "	6'37"	4'48"
		7	215	0.6 serum	12'25"	Nil
		8	206	1.0 "	14'35"	3'50"
		9	215	" "	20'	5'15"
		9a	220	" "	" "	6'40"
		10	200	" "	40'	48'
		10a	205	" "	" "	Very depressed
		11	207	2.0 "	56'	3'20"

The total time represents that from start of drawing blood until the end of injection. The blood for Nos. 1 to 4 was drawn each time by heart puncture, and at once injected.

The rabbit was, therefore, bled into a carotid pipet (65 seconds) and a portion of the blood was centrifugated at once at 3000 revolutions for 3 minutes; the resulting clear plasma was then injected into Nos. 5 and 6. It is to be noted that this plasma killed in dose of 0.6 c.c., an amount which corresponds to 1 c.c. of blood, previously found to be fatal (No. 3). This injection was made 6 minutes after the start of bleeding; the plasma was perfectly fluid, and an unused portion did not clot until 3 minutes after the injection of Nos. 5 and 6. In this case the observed toxicity of the plasma cannot, therefore, be ascribed to pre-coagulation change, but rather indicates the presence

in the blood, at the moment of withdrawal, of a preformed poison, or, what is more likely, a substance which induces poison production in the recipient.

A second portion of the blood was at once whipped with the glass rod for 3 minutes, at the end of which time a heavy clot began to form. This blood was then centrifugated at 3000 revolutions for 3 minutes. It was intended to test this serum at once, that is, within 6 minutes after the start of bleeding, in order to compare the toxicity with that of the plasma, but this was not feasible and the serum was kept 5 minutes before being tested. It will be seen from test No. 7 that this serum in dose of 0.6 c.c. had no effect, while 1 c.c. was fatal. Apparently, even in this short time, a slight decrease in toxicity as compared with that of the plasma had occurred.

TABLE 98
COMPARATIVE TOXICITY OF A NORMAL RABBIT'S HEART-BLOOD AND SERUM

Donor		Recipient		Intravenous Injection (c.c.)	Total Time*	Result
No.	Weight	No.	Weight			
10	2275	1	200	2 blood	15"	5'40"
		2	205	" "	"	5'
		3	205	" "	20"	3'10"
		4	210	1 serum	47'	Very slight
		5	205	1.5 "	52'	Slight
		6	189	1.75 "	58'	Very slight
		7	180	2 "	1 hr. 4'	Good shock
		8	215	3 "	1 " 12'	Slight
		9	190	3.5 "	1 " 36'	"
		10	205	4 "	1 " 46'	Moderate
		11	195	5 "	1 " 55'	1 hr. 30 min.
		12	207	7 "	2 " 19'	47'

* The total time is that from start of drawing blood until the end of injection.

For Tests Nos. 1 to 3, the blood was drawn, each time by heart puncture; the rabbit was then bled into a carotid pipet, the blood subjected to rod defibrination, then centrifugated at 8000 r.p.m. for 5 minutes. The time for Nos. 4 to 12 is counted from the start of bleeding.

After the removal of the partially defibrinated blood for the tests just given, the remainder was whipped till completely defibrinated (10 minutes), then centrifugated and tested, in pairs, at 20 minutes from the start of bleeding. In 1 c.c. dose, this serum was acutely fatal, as was the serum used in test No. 8. Another pair of tests were made 20 minutes later, the serum being kept in the room; the animals showed great depression, but no spasms or other acute symptoms; one recovered, while the other died after a long delay. Consequently, this serum appears to have lost about half of its original toxicity in the intervening

short time. The possibility that the two test animals were highly resistant must be borne in mind.

A somewhat similar experiment to that given above will be found in Table 69; the blood of the rabbit was inherently toxic in dose of 2 c.c., a smaller amount not being tested. On whipping, the blood defibrinated very slowly, after which it was centrifugated at 3000 revolutions; when tested at 18 minutes from the start of bleeding, it caused acute death in dose of 1 c.c., corresponding thus to tests Nos. 9 and 9a of Table 97.

Another like experiment was made with rabbit No. 3 of Table 69. After the first 2 tests which showed that its blood was inherently toxic in dose of 2 c.c., it was retested 3 days later, when its blood was found to be as toxic as before (Table 98, No. 3). It was then bled into a carotid pipet and the blood defibrinated with the rod; this took place very slowly, requiring about 8 minutes. The blood was then centrifugated at 8000 revolutions for 5 minutes and because of this high speed treatment it was not possible to make the first test until 47 minutes after the start of bleeding. It will be seen that at this point, which nearly corresponds to that of Nos. 10 and 10a of Table 97, 1 c.c. of the serum was practically without effect. A rapid series of tests with progressively increasing amounts of serum showed that very little of the original toxicity was left; a very delayed death ($1\frac{1}{2}$ hrs.) was obtained with 5 c.c., and even 7 c.c. required 47 minutes to produce a fatal result.

It is to be noted that while the heart blood was acutely fatal in dose of 2 c.c., the toxicity of the serum rapidly dropped off. It may be questioned whether this change was influenced by the prolonged defibrination, or by the use of the high speed centrifuge, or was merely due to the incident of time which, as seen from the table, ranged from 47 minutes to 2 hours and 19 minutes. The latter factor is probably the one which obtains.

A further experiment bearing upon the question at issue is presented in Table 99. For this series of tests a rabbit was bled into a carotid pipet, the bleeding time being 2'24"; of this whole blood, a portion of 3 c.c. was injected at once into guinea-pig No. 1, without any effect following. Unlike the bloods used in the 2 preceding tables, this was clearly non-toxic. The blood was then whipped for 5 minutes, and the resulting defibrinated blood was tested on No. 2 with fatal result, the toxicity being that developed incidental to the clotting

process. It was finally centrifugated at 3000 revolutions for 5 minutes, and the clear serum in dose of 3 c.c. was tested, 25 minutes after starting to bleed, with no effect. It will be seen from the table that while 3 c.c. of defibrinated blood (approximately 1.8 c.c. serum) sufficed to kill when injected 9 minutes from the start of bleeding, 6 c.c. of serum were necessary at 34 minutes. In other words, the defibrinated blood was more than 3 times as toxic as the cell- and suspenoid-free serum. The dose of 6 c.c. represents an average lethal dose and not a fatal one, as can be seen from tests Nos. 7 and 8, made after the serum was incubated at 37 C.

It would seem that, as in the case of anaphylatoxin production by agar, etc., the maximal toxicity is developed in the few seconds immediately preceding and following the clot formation. This rapid rise is

TABLE 99
COMPARATIVE TOXICITY OF A NORMAL RABBIT'S BLOOD AND SERUM, BEFORE AND AFTER
DEFIBRINATION

Guinea-Pig			Total Time*	Result
No.	Weight	Intravenous Injection (c.c.)		
1	200	3 whole blood	3' 6"	Nil
2	210	" defibr. "	8' 51"	5'
3	204	" serum	25'	Nil
4	178	6 "	34'	2' 51"
5	203	4 "	37'	Slight
6	190	5 "	42'	Very slight
7	200	6 " (15', 37 C.)	62'	Practically nil
8	205	6 " (30', ")	78'	8'

* The total time is from the start of bleeding into carotid pipet until end of injection.

followed by a fairly rapid fall, but the toxicity does not reach the low level of that of circulating plasma which can be considered as nil in the strictly normal rabbit, one whose blood is not inherently toxic. This drop is less complete if the serum remains in contact with the clot than if it is removed from such contact by centrifugation. Evidence substantiating this statement will be presented later on in the paper.

The cause of this rapid drop in toxicity was at first rather difficult to explain, since anaphylatoxic sera, in general, retain their poisonous quality even after long incubation. It is clearly associated with the removal of the suspended clot and cells. The highly toxic condition must be correlated with the sudden physical disturbance which occurs in the plasma just before, and during coagulation. The visible disturbance, that is, coagulation, is soon at an end, but the other dis-

turbance, that of toxicity, may persist for some time. The immediate cause of the coagulation of blood is the formation of an accelerator, commonly known as the fibrin ferment, which reacts with fibrinogen; likewise, the direct cause of the toxicity must be sought in a similar catalyzer which reacts with some very labile blood constituent. As a result, a certain amount of poison is produced, and this is in solution, since it remains in the serum after centrifugation; the greater part of the observed toxicity of whole, or of defibrinated blood is not due to such soluble poison, but rather to the accelerator which is injected with the blood into the animal.

TABLE 100

THE IN VIVO PRODUCTION OF ANAPHYLATOXIN IN RATS INJECTED WITH NORMAL RABBIT BLOOD (A), OR SERUM (B); TRANSFUSION TO GUINEA-PIGS

Series	Donor (rat)			Recipient (guinea-pig)		Total Time*	Transfer Time†	Result
	No.	Weight	Blood Injected (c.c.)	Weight	Rat Blood (c.c.)			
A	1	160	3 blood	210	2	1'15"	20"	Slight
	2	180	" "	185	"	1'12"	18"	"
	3	175	" "	195	"	2' 3"	28"	Near-kill
	4	200	" "	175	"	2'40"	31"	5'54"
	5	200	" "	170	"	3' 4"	34"	4'20"
	6	185	" "	175	"	4' 5"	25"	3'50"
	7	185	" "	210	1	4'15"	50"	Very severe
	8	125	" "	205	1	4' "	20"	Slight
	9	210	1.5 "	190	2	2'55"	29"	Moderate
B	10	140	10 serum	183	2	8'45"	25"	Nil
	11	130	" "	204	2	4'20"	30"	Slight
	12	190	15 "	206	3	5' "	35"	Very slight

* Total time is that from start of injection of rat until end of injection of guinea-pig. The time taken for the transfusion from the rabbit to the rat is not given, but it ranged from 2'25" to 3'20"; this is time from moment of starting to draw blood from the rabbit until end of injection of the rat, and was selected so as to allow pre-coagulation toxicity to develop in the rabbit blood.

† The transfer time means the interval from the entrance of the syringe into the heart of the rat until its withdrawal from the guinea-pig. It therefore represents the maximal time any of the rat blood was in the syringe, and it must be kept as short as possible.

The difference between the total and transfer time represents the reaction period during which anaphylatoxin may be made within the rat.

It has been pointed out in connection with Table 96 that the white rat is about as susceptible as the guinea-pig to the pre-coagulation toxicity. This clearly rules out of consideration such anaphylatoxin as may have been already developed in dose of blood injected, since the rat can withstand enormous doses of anaphylatoxin without any ill effect. If the toxicity of blood is associated with anaphylatoxin-production, it follows that the fatal results in rats must be due to the injection of an accelerator which then reacts with the large volume of

blood within the animal, and thus gives rise to the anaphylatoxin which causes death.

The effect of this accelerator upon the blood plasma is analogous to that of agar, peptone, kaolin, silicic acid, bacteria, trypanosomes, etc. These substances or cells, strictly speaking, are not toxic in themselves, but they induce a disturbance within the plasma or serum. This may be demonstrated in fresh blood by the method devised by Professor Novy and used first in the analysis of the action of several of the above agents. The results of the application of this method will be found in Table 100.

Inducing Power of Fresh, Undefibrinated Rabbit Blood.—Of all the sera that have been tested, that of the rat yields by far the highest toxicity when ordinary procedures for anaphylatoxin production are applied. On the other hand, the rat is very resistant to the action of anaphylatoxin, whether this is made from its own or from a heterologous serum. This high lability of rat serum allows one to demonstrate poison production, in vivo, by introducing the disturbing substance into the circulation of the rat, and then rapidly transfusing its blood into a guinea-pig.

For the tests given in Series A of Table 100, the rabbit blood was obtained by heart puncture, and then kept in the syringe for a varying length of time. This was 2 minutes for Nos. 1, 3, 4, 5, 9, and 3 minutes for Nos. 2, 6, 7, and 8. One rabbit was used for Nos. 1 and 2; another for Nos. 6, 7, 8; and a third for Nos. 3, 4, 5, and 9. After being thus kept in the syringe in order to reach the precoagulation stage, the blood was then injected into the femoral vein of large white rats, the dose being 3 c.c., except in the case of No. 9. Within a minute after such injection the rats usually showed very severe symptoms: respiratory trouble, dyspnea, and spasms. After the injection each rat was kept on the board and at a given period the heart was exposed and 2 c.c. of blood were withdrawn as rapidly as possible and at once injected into a guinea-pig. This transfer time was necessarily a trifle long on account of the marked drop in blood pressure of the rat; it was because of this difficulty that only 1 c.c. was transferred in the case of Nos. 7 and 8.

It is to be noted that, as in like transfusions after injection of agar (Table 65), or of peptone (Table 92), the result was slight in tests made with rat blood having a reaction time of less than a minute (Nos. 1 and 2), that is, where the blood was drawn before any symp-

toms had appeared. But as the reaction time, that in which the rabbit's blood remained in the rat's circulation, increased so did the severity of the effects in the guinea-pig; the effects were those of typical anaphylactic shock with typical findings in case of death. Careful examination of the guinea-pig heart and large vessels failed to disclose thrombi. It must be concluded, then, that the rabbit blood, when injected in the preclot stage into the circulation of the rat, renders the blood of the latter toxic for guinea-pigs. Such rabbit blood is not only poisonous, but also possesses an inducing power whereby the blood of the recipient is rendered toxic.

Inducing Power of Rabbit Serum.—Does this inducing activity persist after defibrination and centrifugation? It was very important to ascertain whether the inducing power, found to be present in the whole blood, persisted or was absent from the serum. To test this, experiments similar to the preceding were carried out, differing only in the substitution of rabbit serum for preclot blood. The results of such tests will be found under Series B, Table 100.

For test No. 10, a normal rabbit serum was employed, which, in dose of 3 c.c., was fatal to 2 of 3 guinea-pigs. Consequently, the rat which received 10 c.c. of this serum was given 3 guinea-pig lethal doses, without showing the slightest effect; the transfusion of 2 c.c. of blood from its heart likewise had no effect.

For tests Nos. 11 and 12, a rabbit serum was used which had been toxified by agar so that the lethal dose was 2 c.c. The two rats received 10 and 15 c.c. of this serum, respectively, which amounts correspond to 5 and $7\frac{1}{2}$ guinea-pig lethal doses of anaphylatoxin; they showed some respiratory disturbance and dyspnea. From the heart of each, 2 or 3 c.c. of blood were withdrawn and injected into guinea-pigs with practically no effect. As illustrating the effect of anaphylatoxin upon the coagulability of blood, it may be added that the blood remaining in the heart of these rats was perfectly fluid 6 minutes after the withdrawal of the test portion.

A comparison of the experiments in Series A and B shows a strikingly different picture. Large amounts of rabbit serum, representing 3 to $7\frac{1}{2}$ guinea-pig lethal doses, do not have the power to make the rat blood toxic, in vivo. The latter series serves, in a way, as control for the former; thus, it will be seen that with the same transfer time as used for Nos. 4 to 6, the transfusion of 2 or 3 c.c. of rat blood was without effect. For other control tests, showing the effect of the

transfusion of normal rat blood into guinea-pigs, reference is made to Table 66. Similar transfusion experiments will be found in Tables 18, 65, 92, and 134.

Apparent Variation in Toxicity of Normal Rabbit Serum.—The defibrination of rabbit blood by means of the glass rod failed to produce sera which were uniformly toxic for guinea-pigs. Although at rare intervals a serum that killed in 3 c.c. dose was obtained, usually the lethal dose of serum prepared by this method averaged 6 c.c. It was also noted that the toxicity of normal rabbit serum apparently was not constant, but seemed to fluctuate above and below a given fatal dose, just as in the case of anaphylatoxin prepared in diverse ways. This variation can easily be shown by testing a sample of serum at intervals of 15 minutes. Care must be taken, however, not to use too large a dose, since otherwise one would obtain an almost unbroken series of fatal results.

TABLE 101

APPARENT VARIATION IN TOXICITY OF NORMAL RABBIT SERUM, KEPT AT 38 C.; SUBACUTE DEATHS

Guinea-Pig		Serum		Result
Number	Weight	Intravenous Injection (c.c.)	Time at 38 C. (hr.)	
1	200	6	—	Slight
2	190	"	$\frac{1}{4}$	8' 5"
3	175	"	$\frac{1}{2}$	5' 40"
4	205	"	$\frac{3}{4}$	Practically nil
5	190	"	1	11'
6	210	"	$1\frac{1}{4}$	8' 15"
7	207	"	$1\frac{1}{2}$	Very slight

The first injection was made 28 minutes after start of bleeding, at which time the serum was placed at 38 C. The serum of one rabbit was used for all of the tests.

For the experiment given in Table 101, a rabbit was bled from the carotid; the blood was defibrinated with the glass rod for 5 minutes, then centrifugated at 8000 revolutions for 2 minutes; the resulting serum was pipetted off and a portion of 6 c.c. was tested at once, the balance being placed at 38 C. and tested at intervals shown in the table.

The subacute deaths in these tests indicate that the serum was but moderately toxic. It is noteworthy that the onset of symptoms was delayed for 2 to 4 minutes. The shock was typical of anaphylatoxin, as were also the autopsy findings. The absence of clot in the heart, the examination being made from 3 to 7 minutes after death, is deserving of special note. The results of this series of tests will be more evident by inspection of Chart XII.

The apparent variation in toxicity in this and similar experiments at first seemed to be too regular to be ascribed to a varying susceptibility of the test animals. In due course of time, however, it was realized that the guinea-pig was by no means as uniform a reagent as had been believed; hence the variation here, and in like tests, must be attributed to this factor and not to a change in the poison. Many experimenters in the past have drawn the erroneous conclusion that the toxicity of a serum had disappeared when one or two tests gave negative results. Marked variation in animals must be expected and abundant evidence of this will be presented.

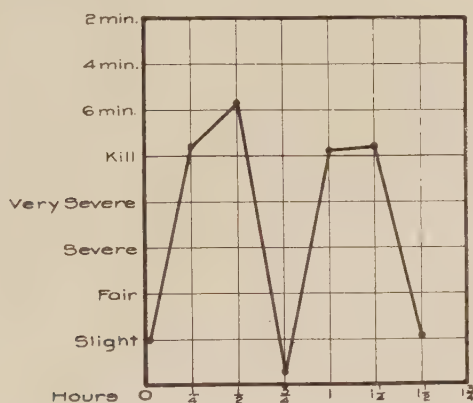


Chart 12. Apparent variation in toxicity of normal rabbit serum (Table 101).

Effects Produced by Normal Rabbit Serum.—While according to Doerr and Weinfurter,³¹ a lethal dose of 6 c.c. for 200 gm. guinea-pigs is high, Mita and Ito³² found 4 of 16 normal rabbits to yield sera which killed in dose of 2 c.c., moreover, they obtained 1 which was fatal in dose of 1 c.c. per 200 gm. Considerable effort was devoted to ascertaining the reason for this marked difference in results. Prepared by thorough rod defibrination, the sera gave results that were very disappointing. Occasionally, a serum could be obtained which was fatal in dose of 3 c.c. (Table 103), but this was the exception, rather than the rule. A year later, however, the problem was solved by the discovery that seemingly perfectly normal rabbits could possess a highly toxic, circulating blood. A striking example of this has been presented in Table 97, where it will be seen that even 1 c.c. of blood may be acutely fatal, and that the serum from such blood may also kill in this

dose. This, and like instances, show that the sera of normal rabbits may vary greatly in initial toxicity. It will be shown, however, that the method of defibrination exerts a marked effect upon the toxicity of a serum.

In the course of the early attempts to increase the toxicity of normal rabbit serum, 2 types of death were found. In one type, which was very acute, the animals succumbed in from 2 to 3 minutes, while in the second, or subacute type, death was delayed. In the latter, the appearance of the symptoms was usually retarded for from 2 to 4 minutes, and death occurred in from 8 minutes to 1 or 2 hours. In both types, the train of symptoms leading to the fatal result was the same. On the other hand, the findings at autopsy were different.

TABLE 102
APPARENT VARIATION IN TOXICITY OF NORMAL RABBIT SERUM, KEPT AT 38 C.; ACUTE DEATHS

Guinea-Pig		Serum		Result
Number	Weight	Intravenous Injection (c.c.)	Time at 38 C. (hr.)	
1	200	6	—	3/30"
2	175	"	1/4	2/35"
3	200	"	1/2	2/45"
4	182	"	3/4	2/55"
5	185	"	1	Slight
6	195	"	1 1/4	3/30"
7	215	"	1 1/2	Slight

The first injection was made 28 minutes after start of bleeding, at which time the serum was placed at 38 C. The serum of one rabbit was used for these tests.

In the rapid deaths, the lungs were found to be enormously distended and very light-colored. One rarely met with any congestion or even with ecchymoses. Further, the right heart almost invariably contained clots when an acutely fatal dose of 6 c.c. was injected. In the slower type the heart clot was absent; the lungs were also greatly distended, but instead of being white they were peppered with ecchymoses, which usually were present in greater number, the slower the death. Such lungs were also edematous. This condition reveals itself, just before death, by the exudation of large amounts of foamy fluid from the nose and mouth of the dying animal.

Jonesco-Mihaiesti³⁴ described a similar difference when large and small doses of rabbit antishoop red cell serum were injected into guinea-pigs. The first effect mentioned he obtained with large doses (5 to 6 c.c.), and the second with doses of 2 c.c. of the serum.

³⁴ Compt. rend. Soc. de biol., 1913, 74, p. 1414.

The tables which follow will indicate some of the variations met with when the same dose of different sera is used, and also the variations in picture that can be produced by employing variable doses of one serum. A series of rather slow deaths are given in Table 101; these results may well be compared with those in Table 102.

For the experiment given in Table 102, the serum of a rabbit was prepared in exactly the same way as for the tests in Table 101. The tests were likewise made under corresponding conditions, the first injection being given 28 minutes after the start of bleeding. It will be seen that the serum of this rabbit was more toxic than that used in the preceding series. This is evident from the larger number of deaths and the acuteness of the shocks. The onset of symptoms was immediate,

TABLE 103

TOXICITY OF A NORMAL RABBIT SERUM, KEPT AT ROOM TEMPERATURE; RELATION OF DOSE TO CLOTTING

Guinea-Pig		Serum		Result	Clot at Autopsy
Number	Weight	Intravenous Injection (c.c.)	Time at Room Temperature (hr.)		
1	200	6	—	3'	+
2	180	"	$\frac{1}{4}$	2'40"	++
3	177	"	$\frac{1}{2}$	2'45"	+++
4	171	"	$\frac{3}{4}$	2'	+
5	177	"	1	3'10"	++
1a	182	3	—	6'40"	0
2a	179	"	$\frac{1}{4}$	42'	0
3a	195	"	$\frac{1}{2}$	50'	0
4a	174	"	$\frac{3}{4}$	Very slight	—
5a	190	"	1	3'15"	++

The sign + indicates a very slight clot; ++, a fair one; and +++, much clot in heart at autopsy.

and the shock was typical of anaphylatoxin poisoning. A further and more striking instance of marked toxicity of a normal serum is given in Table 103.

Relation of Dose to Picture at Autopsy.—For the series of tests in Table 103, a rabbit was bled in the usual way, and, after rod defibrination, the blood was centrifugated at 8000 revolutions for 5 minutes. Tests Nos. 1 and 1a were made 50 minutes after start of bleeding; the other tests were made at quarter hour intervals, the serum being kept in the dark and at room temperature instead of at 38 C., as in the 2 preceding sets. The serum of a single rabbit was used for all of these tests.

The serum was toxic in dose of 3 c.c., and on keeping, it seemed to become less poisonous (Nos. 2a to 4a) ; test No. 5a, however, was more acutely fatal than the first, probably because the guinea-pig was more than usually susceptible, though of course it is possible that Nos. 2a to 4a were somewhat resistant. On the other hand, the 6 c.c. which represents about 2 average lethal doses, always produced acute death with more or less clot in the heart. The same serum, in 3 c.c. dose, produced no intracardiac thrombosis except in the case of No. 5a.

Other instances corresponding to test No. 5a were found where a dose of 3 c.c. of serum caused death and the heart showed a slight clot at autopsy, but such results were rather exceptional and imply either marked response on the part of the animal, or that more than one lethal dose was contained in the serum injected. It must not be inferred, however, that rapid postmortem clotting is due to much

TABLE 104
COMPOSITE TABLE SHOWING THE RELATION OF DOSE OF RABBIT SERUM TO TIME OF DEATH
AND TO THE PRESENCE OR ABSENCE OF CLOT

No.	Serum (c.c.)	Time of Death	Clot	No.	Serum (c.c.)	Time of Death	Clot	No.	Serum (c.c.)	Time of Death	Clot
1	6	8'	+++	9	6	4' 5"	0	17	3	4'10"	⊕
2	"	2'30"	+++	10	"	4'56"	0	18	"	3'40"	0
3	"	1'50"	+++	11	"	8' 5"	0	19	"	4'30"	0
4	"	3'20"	+++	12	"	5'45"	0	20	"	16'20"	0
5	"	3'	+++	13	"	11'10"	0	21	"	3'25"	0
6	"	2' 5"	+++	14	"	8'25"	0	22	"	7'	0
7	"	2'10"	+++	15	"	7'55"	0	23	"	3'20"	0
8	"	3'45"	+	16	"	7'	0	24	"	6'40"	0

All injections were given intravenously. The sign ⊕ indicates a doubtful clot.

poison, but rather that it is due to an increased amount of accelerator or thrombin. It has been shown in Part V that many multiple doses of anaphylatoxin may be injected without causing clot formation; indeed, the reverse occurs. In view of this fact, it follows that early intracardiac clotting in deaths caused by serum is due to a residual inducing power, such as has been demonstrated in connection with whole blood. The injection of such blood into rats and guinea-pigs gives rise to an early clot.

The fact that large doses produce death with clot while smaller doses may kill without clot was noted by Doerr and Moldovan,²⁴ while working with rabbit immune serum. They did not attribute the death of such animals to thrombosis. In all of the guinea-pigs of this series, the lungs showed maximal distention; in the slow deaths the lungs

were peppered with hemorrhagic spots, while in the rapid cases they were pale.

By way of illustrating the constancy of the effects shown in Table 103, the results of a number of experiments are collated in Table 104. These were obtained with a number of different sera, tested on various occasions.

Antagonistic Action of Sodium Oxalate.—It has been shown that a rabbit serum which, in dose of 6 c.c., produces death with intracardiac clotting may be expected to kill, in a dose of 3 c.c., but without thrombosis of the heart and large vessels. It might be expected that by mixing such serum with sodium oxalate solution, a dose of 6 c.c. would cause death without the formation of clot. Experiments made with this object in view were successful as shown in Table 105.

TABLE 105
ANTAGONISTIC ACTION OF SODIUM OXALATE; CLOT PRODUCTION AND TOXICITY

Exper.	Guinea-Pig		Given Mixture of		Contact Time (min.)	Result	Clot
	No.	Weight	Serum (c.c.)	Oxalate (c.c.)			
A	1 C*	196	6	—	—	3'	++
	2	182	"	1	3	2'50"	0
	3	203	"	0.5	"	2'50"	++
B	4 C	198	"	—	—	3'35"	++
	5	182	"	0.75	3	1'45"	0
	6	205	"	1	6	3'	0
	7	201	"	1	9	Moderate	—
	8 C	184	"	—	—	3'15"	+++
	9 C	204	3	—	—	3'15"	0
	10 C	194	6 salt	1	—	Nil	0
C	11 C	180	6	—	—	4'	+++
	12	189	"	1	3	3'50"	+++
	13	200	"	1	8	3'	0

* The letter C after number implies that the test is a control.

In the experiments given in this table, 3 different normal rabbit sera were employed, and each killed in 6 c.c. dose with intracardiac clot formation. This amount of serum was mixed with varying quantities of a 1% sodium oxalate solution (in 0.85% salt solution) and allowed to stand at room temperature, the duration of contact being indicated in the table; the mixture then was injected intravenously. The fine precipitate of calcium oxalate that formed at once upon the addition of the oxalate is not a factor in the death of the animals, since its removal by centrifugation does not in any way affect the result. When centrifugation was employed, it necessarily lengthened the contact time

for the mixture (tests Nos. 6, 7, and 13). It may be added that control tests with 1 or 2 c.c. of oxalate diluted with salt solution had no effect. The autopsies were made as always, 3 minutes after death.

The serum employed for Exper. A, as shown by control (No. 1), killed in dose of 6 c.c. with clot formation. This serum was also fatal in 3 c.c. dose, but in that case no clot resulted; 1 c.c. of oxalate prevented clot formation, while 0.5 c.c. did not do so.

In Exper. B, where a different serum was used, controls Nos. 4 and 8 show that in 6 c.c. dose it killed with presence of clot, while 3 c.c., though fatal, caused no clot (No. 9). Here again it will be seen that 0.75 and 1 c.c. of the oxalate, while not preventing death did do away with clot formation (Nos. 5 and 6). Test No. 7 produced only a moderate shock, the contact time in this case being 9 minutes. Subsequent control tests (Nos. 8 and 9) showed that the serum was as active as in the beginning. The harmlessness of an oxalate salt mixture is shown in control test No. 10.

In Exper. C, which was made several days later, the serum killed in dose of 2 c.c., without clot, while in dose of 6 c.c. it was fatal, with clot production (No. 11). This amount of serum, therefore, represents 3 lethal doses; when mixed with 1 c.c. of oxalate, a contact of 3 minutes did not prevent clot, whereas one of 8 minutes did.

The above results indicate that an oxalate-serum mixture, with a contact of 3 minutes, may kill without clot, provided the amount of serum does not represent more than 2 lethal doses. If the toxicity is greater than this, a longer contact appears to be necessary. And, further, with a sufficiently long contact the serum may become non-fatal. It would seem as if the oxalate exerted an antagonistic action, first, as to the clotting factor, and second, on the toxicity.

In this connection it may be stated that a fresh oxalate plasma which was nontoxic in dose of 3 c.c., became acutely fatal in the same dose when tested within a few minutes after recalcification. In this case the oxalate inhibited the toxicity as well as the coagulation. A further illustration of this action of oxalate is seen when rabbit blood, drawn from the heart and kept in the syringe for 3 to 3¼ minutes, is added to a sodium oxalate solution. As shown in Table 96, such whole blood, in a dose of 3 c.c., will cause either a severe shock or an acute death; the oxalate mixture, however, does not clot and is not toxic. Blood thus oxalated, when injected at 5 and at 11 minutes after start of bleeding, was without effect (3.3 c.c.).

Effect on Toxicity of Spontaneous Clotting: Minimal Contact with Clot.—Most variable results, as to toxicity, were obtained when the blood was defibrinated by means of the glass rod. As a rule, the fatal dose of the serum thus obtained rarely goes below 6 c.c. On the other hand, as mentioned, Mita and Ito obtained several rabbit sera which were fatal in dose of 2 c.c., and one which killed in 1 c.c. The latter was fatal in 7 tests made from $\frac{1}{2}$ to 5 hours after bleeding. They make no mention of the way in which they clotted the blood for their tests. They do state that the rabbits were bled from the carotid, and that the blood was at once centrifuged and tested immediately. The serum was then kept at room temperature in the dark, and retested at intervals. We were led to infer, therefore, that the blood was allowed to clot spontaneously in the centrifuge tube.

Our first attempt to influence toxicity, by varying the time and kind of clot contact, consisted in trying to duplicate as closely as possible the time relations in Mita and Ito's experiments.

In one experiment the rabbit was bled white in 3 minutes and the blood was transferred at once to 3 centrifuge tubes. Within $8\frac{1}{2}$ minutes after start of bleeding the centrifuge had reached 8000 revolutions, and this speed was maintained for 5 minutes. Under these conditions the clot was removed as fast as formed. Indeed, in one instance the cell-free layer showed a clot at the end of centrifugation. In 22 minutes after starting to bleed, the first injection was made. The serum was then kept in the room and retested as indicated.

The results of this experiment are given in Table 106, A. The effect here was no better than with rod defibrination. Since the toxicity of a serum does not increase when kept at room temperature, the experiment was discontinued.

Other attempts were made with this method of rapid removal of the spontaneous clot, but they were productive of no better result. While serum obtained in this way will not kill in dose of 3 c.c., it will do so regularly with 6 c.c. It was evident from these tests that the cause of increased toxicity must lie in some other direction. An attempt was made to reach a greater degree of toxicity by allowing the blood to remain undisturbed in contact with the fibrin of the spontaneous clot. It seemed possible that such contact with the extensive fibrin surface might influence the result.

Contact for Thirty Minutes with Spontaneously Formed Clot.—The results of an experiment of this kind are given in Table 106, B. The

rabbit was bled as usual into a carotid pipet, the blood was transferred at once to 3 centrifuge tubes, and allowed to clot spontaneously. It remained thus at room temperature for 30 minutes, when it was centrifuged at 8000 revolutions for 15 minutes. The first test of the clear serum was made 72 minutes after the start of bleeding.

The serum thus prepared was fatal in dose of 6 c.c., but 3 c.c. had very little effect. Clearly, a long contact was no better than a minimal one. Possibly a long contact with the fibrin caused a reversion change;

TABLE 106

EFFECT OF SPONTANEOUS CLOTTING ON TOXICITY OF RABBIT SERUM; MINIMAL CONTACT WITH CLOT (A); CONTACT FOR 30 MINUTES (B); CONTACT FOR 20 MINUTES (C)

Exper.	Guinea-Pig		Rabbit Serum		Result
	Number	Weight	Intravenous Injection (c.c.)	Time at Room Temperature (hr.)	
A	1	196	3	—	Moderate
	2	185	6	—	4'30"
	3	208	3	1/4	Very slight
	4	195	6	1/4	3'15"
B	5	180	6	—	2'35"
	6	194	3	—	Moderate
	7	192	6	1/4	3'10"
	8	203	3	1/4	Very slight
	9	191	6	1/2	Severe
	10	201	6	3/4	4'35"
	11	207	6	1	3'40"
	12	192	6	2 1/2	2'40"
	13	200	3	"	Slight
C	14	198	3	—	3'15"
	15	198	2	—	Very slight
	16	175	3	1/4	3'10"
	17	196	3	1/2	Very slight
	18	200	3	3/4	2'50"

In Exper. A, the first 2 injections were made 22 minutes after start of bleeding; in Exper. B, this time was 72 minutes; and in Exper. C, it was 51 minutes. The other tests were made at subsequent intervals, as indicated.

to test this, the experiment was repeated with shorter contact time, about midway between those given in Expers. A and B; hence, a contact time of 20 minutes was taken for the next trial.

Contact for Twenty Minutes with Spontaneously Formed Clot.—An experiment of this kind will be found in Table 106, C. The blood of a rabbit was blown at once into centrifuge tubes and these were left at room temperature for 20 minutes, after which they were centrifuged at 8000 revolutions for 15 minutes. The clear serum was pipetted off

and tested at once, the balance being kept at room temperature and tested at intervals, as shown in the table.

The increase in toxicity was realized, since, as seen from the table, out of 4 injections of 3 c.c. of such serum, 3 resulted in acute and typical death. This serum was used 4 hours later for Exper. A, Table 105, and a similarly prepared serum was employed for Exper. B in the same table; that used in Exper. C, likewise made in this way, killed in dose of 2 c.c. a 181 gm. guinea-pig in 62 minutes, the findings being typical. This experiment was repeated at other times with like result. In only one instance did such serum fail to kill in dose of 3 c.c., the serum in that case being very lipoidal.

To sum up these experiments upon the toxicity of serum obtained from spontaneously clotted blood, we may say that both short (8 minutes) and long contact (30 minutes) fail to produce a very toxic serum. On the other hand, a contact of 20 minutes with the fibrin network yields a serum which quite consistently kills in dose of 3 c.c., and once was fatal in 2 c.c. dose, the highest toxicity which we were then able to secure. At the time this work was done it was believed that the rabbit blood was fairly uniform as to its capacity for poison production, but a year later it was found that such was not the case, and that rabbits could possess an inherently toxic blood, one which, on rod defibrination, was fatal in dose of 1 c.c. (Table 97).

Effect on Toxicity of Bead Defibrination at Room Temperature.—Moldovan,¹¹ testing the toxicity of freshly defibrinated rabbit blood for homologous animals, claimed that a vigorous shaking with glass beads for 5 minutes was necessary to make blood toxic. He stated that the failure of Blaizot³⁵ to detect the toxicity in normal rabbit blood was probably due to the fact that he did not shake it vigorously enough with beads. Studzinski,³³ in testing the effect of fresh homologous blood on dogs, came to the conclusion that energetic shaking with beads was necessary to liberate the toxic substance, which he believed to come from the erythrocytes.

We have tested the effect of bead defibrination upon the toxicity of normal rabbit serum for guinea-pigs, and although we consider the use of beads an important factor, we do not think that very vigorous or very prolonged shaking is of prime importance.

For the experiment given in Table 107, a rabbit was bled white from the carotid, and the blood was blown at once into an Erlenmeyer

³⁵ Compt. rend. Soc. de biol., 1910, 68, p. 1124.

flask of about 500 c.c. capacity, the bottom of which was covered to a depth of from 5 to 6 mm. with glass beads. The flask was then shaken vigorously at room temperature for 2 minutes, at the end of which time the coagulation appeared to be complete. A portion of the blood was then transferred to a centrifuge tube and whirled at 3000 revolutions for 12 minutes; tests Nos. 1, 2, and 3 were made with this serum. The bulk of the blood was left in contact with the beads and clot at room temperature for two hours, when it was centrifuged and the serum used for Nos. 4 and 5.

The serum which remained in contact with the beads, clot and cells (No. 4) was apparently more toxic than that which was not. The failure to kill in Nos. 1 and 3 must be ascribed to the individual resistance of the test animals. At autopsy, slight clots were found in the hearts of both fatal shocks, a result rarely encountered with like dose

TABLE 107
EFFECT OF BEAD DEFIBRINATION, AT ROOM TEMPERATURE, ON THE TOXICITY OF RABBIT SERUM

Guinea-Pig		Serum		Result
Number	Weight	Intravenous Injection (c.c.)	Time at Room Temperature (hr.)	
1	182	3	—	Fair
2	184	"	1/4	7'45"
3	181	"	1 3/4	Slight
4	202	"	2	2'20"
5	180	2	2	Slight

Test No. 1 was made 23 minutes after the start of bleeding.

of serum prepared by rod defibrination. This would indicate that the grinding action of the beads may liberate more of the accelerator than is the case when the rod is used.

Effect on Toxicity of Bead Defibrination at 40 C.—Since the production of anaphylatoxin occurs more readily at the body temperature, or slightly higher, we tested the effect of coagulation at 40 C. Since the production of a toxic serum by the addition of agar occurs with facility at this temperature, it seemed that the same might be the case if fibrin made its appearance in the blood under similar conditions.

The results of 2 experiments of this kind will be found in Table 108. For the first of these, the rabbit blood was transferred at once from the carotid pipet to a flask with beads, which had been warmed for 15 minutes in a water-bath at 40 C. The flask was then shaken in the water-bath for 2 minutes. The larger part of the blood thus defibri-

nated was then centrifuged at 8000 revolution for 5 minutes, while a small bulk was whirled at 3000, in order to secure an earlier test than is possible with the high speed machine. This second portion, designated as A, was therefore tested first, while that centrifuged at 8000 is marked B.

It would appear from the results, as given in Table 108, that the very rapid bead defibrination at 40 C. yields a distinctly nontoxic serum, at least as to the dose employed. A similar unfavorable result was obtained in the second experiment, where the blood was added to the bead flask, previously warmed at 40 C. for 10 minutes; but instead of agitating the flask in the water-bath it was shaken in the room. The

TABLE 108
EFFECT OF BEAD DEFIBRINATION, AT 40 C., ON THE TOXICITY OF RABBIT SERUM

Exper.	Portion	Guinea-Pig		Serum		Result
		No.	Weight	Intravenous Injection (c.c.)	Time at Room Temperature (hr.)	
I	A	1	180	3	—	Very slight
	B	2	189	"	$\frac{1}{4}$	" "
		3	201	"	$\frac{1}{2}$	Nil
		4	185	"	$\frac{3}{4}$	Slight
		5	205	"	1	Very slight
II	A	6	180	"	—	3'30"
		7	210	"	$\frac{1}{4}$	Severe
	B	8	205	"	$\frac{1}{4}$	Slight
		9	178	"	$\frac{1}{2}$	"
		10	182	"	$\frac{3}{4}$	Severe
		11	200	"	$1\frac{1}{4}$	Very slight
		12	189	"	$1\frac{1}{2}$	Nil

The time from the start of bleeding to the injection of No. 1 was 16 minutes, while that of No. 6 was 17 minutes.

defibrination was, therefore less rapid than in Exper. 1, and the portion of the blood centrifuged at low speed caused an acutely fatal shock (slight clot in heart); on the other hand, portion B, which was centrifuged at 8000 revolutions for 5 minutes, had but a slight effect, except in No. 10, where a severe shock resulted.

In the preceding experiments, the blood was centrifugated in toto, and the serum freed from contact with the clot as soon as possible. A different result would have been obtained if it had remained in contact with the beads and clot, and at intervals small portions had been removed, centrifuged and tested. This, however, does not improve the result as will be seen on reference to Exper. A, Table 112.

From the standpoint of securing high toxicity, this method must be considered a complete failure. The poor results are not entirely due to the individuality of the rabbits employed, but depend rather on the temperature at which coagulation occurs (Table 112). It would be of interest to know whether, under these conditions, the serum would prove equally inert in 6 c.c. dose. To have a serviceable method which would give with some regularity a nontoxic serum would be a distinct advantage. It will be shown later that blood defibrinated by this method may be quite nontoxic, since 10 and even 12 c.c. may be without action on some guinea-pigs.

Effect on Toxicity of Chilled Bead Defibrination.—The failure to obtain the desired increase in toxicity by defibrination with beads at 40 C., and the fairly good results secured by spontaneous clotting suggested trials with chilled beads. It is well known that the chilling of freshly drawn blood retards to a considerable extent the progress of coagulation. It was possible that the state of fibrinogen, as it changes from its dispersed form to the coarser aggregates that make up the fibrin, was as important to the initiation of the poison-formation as that of the agar, or other suspenoid used in anaphylatoxin production. If such were the case, by retarding the coagulation and thus keeping the aggregating fibrin or threads in the finest possible state, results as good, if not better than those obtained by spontaneous clotting with sera, might be expected. Hence, the attempt was made to bring on the clot slowly by exposure to cold. It will be seen from the following experiments that the slow coagulation of rabbit blood is an important factor in toxification.

For the experiment shown in Table 109, a flask containing the usual layer of beads was iced for 30 minutes. A rabbit was then bled into a carotid pipet (3 minutes) and the blood was at once blown into the iced flask, which was then allowed to stand at room temperature for 5 minutes. At the end of this time, a beginning coagulation showed itself in the form of a slight gel. This was easily broken up by gentle swinging for 2 minutes, the clot collecting upon the beads. The flask, with the blood in contact with the fibrin-coated beads, was then kept at room temperature, and, at intervals, portions of 12 c.c. were drawn off, centrifuged at 3000 revolutions, and the serum thus obtained was tested for its toxicity. Seven portions were thus centrifuged; these are indicated in the table by the corresponding numbers. The results of this experiment should be compared with those given in Table 113.

This method gave the best results yet obtained. That it was not a matter of chance, due to an individual peculiarity of the rabbit used, will be shown in comparative tests to be presented later. An inspection of the table shows that this serum killed rapidly in dose of 3 c.c., while that of 2 c.c. was acutely fatal once, and caused very severe shocks in 3 of 4 other tests. The variable resistance of guinea-pigs accounts for the slight effects seen in Nos. 5 and 6. In 2 experiments, similar to that of Table 109, the serum tested 17 minutes after start of bleeding proved fatal in dose of 2 c.c. In one of these the toxicity, tho sub-acute, persisted through 4 consecutive tests made during the first hour. In this experiment the shaking with the beads was very gentle, yet contrary to the idea of Moldovan,¹¹ the toxicity was extremely high.

TABLE 109
EFFECT OF CHILLED BEAD DEFIBRINATION ON THE TOXICITY OF RABBIT SERUM

Guinea-Pig		Serum		Result
Number	Weight	Intravenous Injection (c.c.)	Time* at Room Temperature	
1	185	3	18 min.	2'15"
1a	179	2	22 "	Severe
2	185	3	28 "	2' 5"
2a	178	2	21 "	3'45"
3	182	2	40 "	Severe
4	178	2	1 hr. 9 min.	Near-kill
4a	182	1.5	1 " 11 "	Slight
5	184	2	2 " 30 "	Very slight
5a	180	3	2 " 33 "	Severe
6	190	3	3 " 9 "	Very slight
7	176	3	4 " 36 "	4'

* The time given for each test is that from the start of bleeding.

The pooled sera from the several portions (Nos. 1 to 4) used in the above experiment were subsequently treated with agar by the sol-gel method in order to see if such initially toxic serum could be toxified still more. The agar-serum mixture was placed at 37 C., and portions were removed, centrifugated and tested consecutively at $\frac{1}{4}$, $\frac{1}{2}$ and 1 hr., respectively. The first portion, in dose of 2 c.c., gave a very severe shock; the second and third portions in like dose killed in 4'50" and 2'35", respectively, while a test of 1 c.c. after incubation for 1 hour gave a severe shock. A similar test experiment made with the serum used in Exper. C, Table 105, also gave a 2 c.c. lethal dose after

incubation for 45 minutes at 37 C. In a third attempt, the serum had been prepared by spontaneous clotting (20 minute contact) and was toxic in 3 c.c. dose; treated by the sol-gel method (Mixture No. 6), and incubated at 37 C. for 15 minutes, it caused typical shock and death in 5 minutes in dose of 2 c.c. It appears, therefore, that the high initial toxicity of rabbit serum can be slightly increased by treatment with agar.

Comparative Toxicity: Rod and Bead Defibrination.—On account of the possible and actual variation in the quality of the blood in different rabbits, a strict comparison of the two methods of defibrination cannot be made with sera from bloods of different origin. It is essen-

TABLE 110
COMPARATIVE TOXICITY OF SERUM FROM ONE RABBIT: PORTION A, DEFIBRINATED WITH ROD;
PORTION B, WITH CHILLED BEADS. CHILLED ROD SERUM (C)

Exper.	Guinea-Pig		Serum		Result
	Number	Weight	Intravenous Injection (c.c.)	Time* at Room Temperature	
A	1	201	3	24 min.	Very slight
	2	175	"	44 "	Nil
	3	194	"	1 hr.	Slight
	4	176	"	1 hr. 24 min.	"
B	5	199	"	27 min.	4' 5"
	6	195	"	41 "	3'30"
	6a	180	2	47 "	67 min.
	7	180	3	1 hr. 4 min.	3'20"
	8	200	"	1 " 27 "	Very severe
C	9	187	3	24 min.	Very slight
	10	210	"	38 "	" "
	11	180	"	52 "	Nil
	12	181	"	1 hr. 10 min.	Very slight

* The time given for each test is that from start of bleeding.

tial that the blood for both methods should be obtained from the same animal and at the same time; moreover, the time relations should be as close as possible. With these conditions in mind, the following experiment was planned and carried out.

As a preliminary control for Expts. A and B of Table 110, 10 c.c. of blood were obtained from a rabbit by direct heart puncture, and injected into a guinea-pig of 210 gm. within 30 seconds after starting to draw the blood (Table 96, No. 8). The effect was but slight, since only a few mild spasms resulted. The dose given represented 6 c.c.

of plasma; consequently, it was to be expected that the serum would have a low normal toxicity.

The same rabbit was then bled white from the carotid and the blood was at once divided into 2 portions: one of which (A) was immediately defibrinated for 5 minutes with the glass rod, while the other portion (B) was blown into a flask with beads previously chilled by being kept outside the window at -7°C . The flask was then allowed to stand at room temperature for 6 minutes until a soft gel began to form, when it was shaken very gently for 2 minutes.

Both portions, A and B, were then kept at room temperature, and at intervals a sample was removed from each and centrifuged at 3000 for 6 minutes. The 2 sera thus obtained were then injected, the difference in time being, at most, 3 minutes. The results of these parallel tests are given in Table 110, and are also shown in Chart XIII.

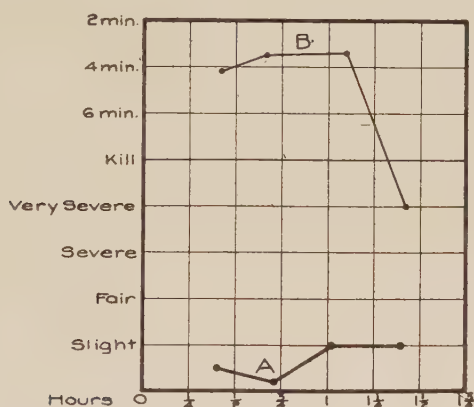


Chart 13. Comparative toxicity of normal rabbit serum: A, rod defibrination; B, chilled beads (Table 110).

This experiment constitutes a perfect comparison of the 2 methods of defibrination. The serum obtained by rod defibrination (A), in four tests, is shown to be practically innocuous in the dose employed. On the other hand, the serum prepared by chilled bead defibrination, from the same blood and under parallel conditions, was very toxic, since in a dose of 3 c.c. it caused 3 acute deaths; even 2 c.c. proved fatal. The conclusion, therefore, to be drawn is, first, that the toxicity of a serum depends on the mode of defibrination; second, that the method of rod defibrination yields a less toxic serum than that of chilled beads.

By way of summary it may be stated at this point that of 14 rod sera, one failed to kill in dose of .6 c.c., in 5 consecutive tests, made at intervals of 15 minutes; 3 of these sera caused subacute deaths, the animals dying as late as 70 minutes. It is evident, therefore, that the rod serum is the least toxic, while that obtained with chilled beads is the most active. The serum from a spontaneous clot, with a contact of 20 minutes, appears to occupy an intermediate position. The occasional poor success with the best method is indicative of individual variation in the rabbit blood.

Effect on Toxicity of Chilled Rod Defibrination.—Inasmuch as it was possible that the poor result with rod defibrination obtained in the preceding experiment was due to the warmer glass surfaces, it appeared desirable to make a control test in which the receiver with its rod was likewise chilled before use.

For this purpose a rabbit was bled through a cannula inserted into the carotid directly into an iced receiver. The latter consisted of a test-tube on foot, plugged with cotton and provided with a glass rod for the purpose of defibrination; before receiving the blood it was thoroughly chilled by being packed in cracked ice. After being filled with blood it was kept in the room for 6 minutes, until beginning coagulation; the blood was then whipped for 4 minutes, till coagulation was complete. The blood thus defibrinated was kept at room temperature, and at intervals portions were removed, centrifuged and tested. It will be noted that contact of the serum with the clot and corpuscles was thus maintained the same as in the previous experiments.

The results are given in Table 110, Exper. C. They are essentially the same as those of Exper. A. Although coagulation was retarded by the chilling of the receptacle, the toxicity was not increased. The explanation for this difference in the action of chilled beads and chilled rod must lie in the physical conditions presented in the 2 methods. In rod defibrination the clot is rolled up into a solid mass, whereas, in the former method, the fibrin is deposited on the beads; this enormous surface of fibrin and glass may be looked upon as a determining factor. It is conceivable that the large soft mass of fibrin which is gathered up by the rod, in being whirled through the blood, removes by adhesion or adsorption the suspended accelerator, and thereby prevents marked toxification of the serum; the removal of such a catalyzing substance would be less complete in the bead method where clotting is retarded and the agitation is of a gentle nature.

Effect on Toxicity of Rapid Clotting with Chilled Beads.—When the blood is caused to clot rapidly in an iced flask containing beads, the toxicity does not reach as high a level as when the clotting is retarded. This rapid clotting can be brought about by bleeding the rabbit slowly into a carotid pipet. In the most successful experiments with chilled beads, the blood was received into pipets which had not been chilled. The operation of bleeding usually lasted from 2 to 3 minutes, and the blood as soon as drawn was blown into the chilled bead flask. If, however, the bleeding operation took from 4 to 5 minutes, coagulation was already beginning to take place when the blood was introduced into the flask. The low temperature of the flask was not sufficient to check the clotting when this had once begun. The serum obtained under these conditions is no more toxic than that obtained by rod defibrination. An experiment of this kind is reproduced in Table 111.

TABLE 111

EFFECT ON TOXICITY OF RAPID CLOTTING WITH CHILLED BEADS: SERUM PORTION A WAS KEPT AT ROOM TEMPERATURE; PORTION B, AT 37 C.

Exper.	Guinea-Pig		Serum		Result
	Number	Weight	Intravenous Injection (c.c.)	Interval (hr.)	
A	1	195	3	—	Fair shock
	2	185	"	$\frac{1}{4}$	Very slight
	3	193	"	$\frac{1}{2}$	Fair
	4	189	"	$\frac{3}{4}$	Moderate
	5	182	"	1	Fair
B	6	173	"	$\frac{1}{4}$	Slight
	7	180	"	$\frac{1}{2}$	Severe
	8	201	"	$\frac{3}{4}$	Slight
	9	173	"	1	Very slight

No. 1 was injected 35 minutes after start of bleed; the others were tested subsequently at intervals given.

For the tests in the table mentioned, a rabbit was bled, 5 minutes elapsing from the start to complete exsanguination. The blood was blown at once into a flask containing beads, which had been thoroughly cooled by being packed in cracked ice. The surface of the blood set into a gel almost immediately after being transferred. The flask was shaken gently for 1 minute, when defibrination was complete. The blood was then transferred to centrifuge tubes and whirled at 8000 revolutions for 5 minutes. The serum thus obtained was tested at once (No. 1), while the remainder was divided into 2 portions: Por-

tion A was kept at room temperature, while portion B was placed at 37 C. The subsequent tests were made at intervals of 15 minutes.

In this experiment, the serum was not in contact with the clot and beads for more than 7 minutes at the time when centrifugation began. This condition may account for the rather feeble toxicity, since fractional centrifugation, as in Tables 109, 110, and 113, has been shown to give a highly toxic serum. However, reference to Table 112 will show that blood which is rapidly clotted with beads at 40 C. does not become toxified, notwithstanding fractional centrifugation. It would seem, therefore, that the large mass of clot formed under the condition of the above experiment removed the activating substance from the serum in much the same way as in rod defibrination.

TABLE 112

COMPARATIVE TOXICITY OF SERUM FROM ONE RABBIT: PORTION A, DEFIBRINATED WITH BEADS AT 40 C.; PORTION B, WITH BEADS AT 0 C.

Exper.	Guinea-Pig		Serum		Result
	Number	Weight	Intravenous Injection (c.c.)	Interval (hr.)	
A	1	174	3	—	Very slight
	2	201	"	1/4	" "
	3	207	"	1/2	" "
	4	190	"	3/4	Nil
	5	210	"	1	Very slight
	6	207	"	1 1/4	Slight
B	7	175	"	—	5'25"
	8	194	"	1/4	4'40"
	9	202	"	1/2	Moderate
	10	205	"	3/4	Very severe
	11	201	"	1	5'20"
	12	203	"	1 1/4	Very slight

The interval from the start of bleeding to the injection of Nos. 1 and 7 was 33 and 30 minutes, respectively; the other tests followed at the stated time.

Comparative Toxicity: Bead Defibrination at 40 and at 0 C.—The experiment summarized in Table 108 indicated that blood which was transferred at once after drawing to a flask with beads that had been kept at 40 C., and then defibrinated at this temperature, did not become highly toxic. To avoid once more the objection that we might be dealing here with an individuality as regards the blood source, the experiment given in Table 112 was devised.

For this experiment a rabbit was bled into a carotid pipet, and the blood was transferred at once to 2 flasks containing beads: One of these (A) had been immersed in water at 40 C.; this flask was at once

gently shaken for 2 minutes when defibrination was complete. The other flask (B) had been packed in crackle ice for some time before addition of blood; hence, coagulation was retarded; the clotting began after the blood had stood in this flask at room temperature for 8 minutes. The flask was then shaken for 2 minutes in the same way as flask A. The 2 flasks were then kept in the room, and at intervals of 15 minutes portions of 6 c.c. were removed from each and centrifugated side by side at 3000 revolutions for 5 minutes, after which each serum was tested, the dose being 3 c.c. Of each blood, 6 portions were thus centrifugated and tested.

The results of this experiment are shown in Table 112 and in Chart XIV. They present a striking demonstration of the effect upon toxicity of slow and rapid coagulation of one and the same rabbit

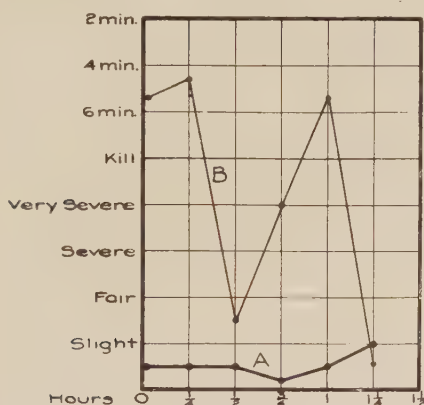


Chart 14. Comparative toxicity of normal rabbit serum: A, beads at 40 C.; B, at 0 C. (Table 112).

blood. The difference here is just as marked as in Expts. A and B of Table 110. The rapid and complete removal of clot, either by rod or by warmed beads, yields a serum of less toxicity than that when chilled beads are employed.

Toxicity of Serum Kept in Contact with Clot.—In a number of experiments recently considered (Tables 109, 110, and 112) the blood was kept in contact with the clot, and portions were centrifuged as needed. The toxicity of a serum thus maintained in contact with its clot is distinctly higher and persists longer than seems to be the case with serum which has been centrifuged out of a blood, *en masse*, and then kept at room temperature. Continued contact of the serum with

either fibrin or a suspended catalyzer at a suitable temperature is needed to develop high toxicity, the condition being exactly similar to that of anaphylatoxin-production by agar or other suspended matter.

The experiment given in Table 113 is intended to show the persistence of toxicity in a serum kept in contact with its clot at room temperature. For this series of tests a rabbit was bled into a carotid pipet (2 minutes) and the blood was transferred at once to a bead flask which had been iced for half an hour. The surface gel appeared in $5\frac{1}{2}$ minutes, whereupon the flask was swung for 1 minute. The blood thus defibrinated was now allowed to stand at room temperature; at intervals of 15 minutes, a portion of 6 c.c. was removed and centrifuged at 3000 for 5 minutes; the resulting serum was then tested in 3 c.c. dose. Ten such portions were tested.

TABLE 113
PERSISTENCE OF TOXICITY IN SERUM KEPT IN CONTACT WITH CLOT; CHILLED BEAD
DEFIBRINATION

Guinea-Pig		Serum		Result
Number	Weight	Intravenous Injection (c.c.)	Interval (hr.)	
1	185	3	—	Very severe
2	180	"	$\frac{1}{4}$	3'25"
3	195	"	$\frac{1}{2}$	4'25"
4	179	"	$\frac{3}{4}$	7'
5	184	"	1	3'20"
6	202	"	$1\frac{1}{4}$	Very severe
7	178	"	$1\frac{1}{2}$	5'15"
8	180	"	$1\frac{3}{4}$	7' 5"
9	187	"	2	4'20"
10	180	"	$2\frac{1}{4}$	4 hr.

The interval from the start of bleeding to the injection of No. 1 was 20 minutes; the other tests were made at subsequent intervals, as given.

The results, as presented in Table 113, show that under these conditions the toxicity remains constant for at least $2\frac{1}{2}$ hours after start of bleeding. The failure to kill acutely in 3 of 10 tests merely indicates greater resistance on the part of the test animal. In view of this high average toxicity, it is probable that the serum would have killed some guinea-pigs in a dose of 2 c.c. The centrifugation of the first portion was commenced 13 minutes after the start of bleeding, which gives a contact time of only about 8 minutes; it is likely that even at this time the full toxicity was developed, though test No. 1 failed to kill.

Toxicity of Serum Freed from Contact with Clot.—It has been pointed out in connection with Table 111 that the early removal of the

serum from contact with clot and corpuscles may explain the feeble toxicity observed in that experiment. It is also possible that the absence of toxicity in such serum was due to the quality of the blood which happened to be used in that experiment. It has been shown in Part III that the reactivity of rabbit serum is subject to considerable variation, and it is not unlikely that this factor holds true for the poison production incidental to blood coagulation.

As a further illustration of the apparent effect of centrifugation on toxicity, the experiment shown in Table 114 is given. It is to be noted that the conditions, except as to centrifugation, are essentially the same as those for the experiment presented in Table 113; the 2 experiments were made on consecutive days, and necessarily the bloods were of different origin.

TABLE 114
APPARENT DECREASE IN TOXICITY OF SERUM AFTER REMOVAL FROM CONTACT WITH CLOT;
CHILLED BEAD DEFIBRINATION

Guinea-Pig		Serum		Result
Number	Weight	Intravenous Injection (c.c.)	Interval (hr.)	
1	192	3	—	3/35"
2	202	"	1/4	Moderate
3	200	"	1/2	Severe
4	185	"	3/4	"
5	205	"	1	Fair
6	201	"	1 1/4	Very slight
7	200	"	1 1/2	Slight
8	195	"	1 3/4	Very severe
9	187	"	2	Slight
10	175	"	2 1/4	Moderate
11	205	"	2 1/2	Nil
12	200	"	2 3/4	Moderate
13	186	"	3	Slight

The interval from the start of bleeding to the injection of No. 1 was 52 minutes; the other tests followed as stated.

For this experiment a rabbit was bled into a carotid pipet (2 minutes) and the blood was blown at once into a bead flask previously chilled in ice. As soon as the clot began to appear on the surface, which was in 5 minutes, the flask was swung for 1 minute. Upon standing for a minute an additional clot appeared, whereupon it was shaken for another half minute. The blood was then quickly transferred to centrifuge tubes and swung at 8000 revolutions for 5 minutes. The clear serum was pipetted off and a portion was tested at once (No. 1); the balance was then kept at room temperature, tests being made at intervals of 15 minutes. The time from the start of bleeding

to the beginning of centrifugation was 25 minutes, and the first test was made at 52 minutes.

It will be seen that of the 13 tests made during the 3 hours following the centrifugation, only the first was fatal. The experiment might therefore be interpreted as showing that a rapid decrease occurred in the centrifugated serum. The validity of such conclusion, however, is doubtful, owing to the absence of a parallel control series, similar to that of Table 113, made with the same blood. Several other trials have shown that the serum obtained under conditions similar to those in the above experiment retained its toxicity for an hour. Still more pertinent is the fact shown in Table 106, No. 12, that the toxicity may persist for 22 hours. In this respect, therefore, the primary toxicity of serum is like that of anaphylatoxin.

Comparative Stability of Serum Toxicity at 37 C., and at Room Temperature.—Mita and Ito⁸² claim that a decrease in the toxicity of normal rabbit serum usually makes its appearance in from 1½ to 4 hours after bleeding. This decrease at times was found to amount to 50%. We have observed that rod sera which killed in 6 c.c. dose in a subacute manner were innocuous in like dose when tested 6 hours later, but the results in general are not very convincing. To interpret a single failure to kill, or even 2 or 3 such negative results, as a permanent decrease in toxicity is not justifiable. It must ever be borne in mind that the guinea-pig presents extreme variations in its susceptibility to anaphylatoxin, and the same holds true for the primary toxicity of a serum. The fact that the first animal of a series is killed, as for example No. 1, Table 114, while the next 12 recover, is far from being evidence of a decrease in toxicity. The more reasonable view is that the fatal case is due to the chance employment of a very susceptible animal; it will be observed in the table mentioned that 3 other guinea-pigs experienced severe shocks. In other words, the amount of poison, in the dose of serum used, was practically sublethal.

Examples of the extreme variation in guinea-pigs have been presented in Tables 45 and 71, and identical conditions exist as to normal sera. The test animal is, therefore, a greater variant than the poison. It is certain that the primary toxicity may persist in a given serum for many hours. Thus, in 3 instances, we found the sera to be just as toxic in dose of 6 c.c. after being kept at room temperature for 22 to 27 hours as they were in the beginning; an instance of this is given in Table 106, test No. 12. Again, as the result of many observations, it

may be said that when a rod serum, in dose of 3 or 6 c.c., causes several deaths during the first hour, it will be found to be equally toxic when retested on a like number of animals at the end of 6 hours, the serum being kept at room temperature.

It was of interest to learn whether a given toxic serum would become less active when kept at 37 C. than the same material held at room temperature. An experiment planned with this object in mind is given in Table 115. A rabbit was bled as usual into a carotid pipet ($3\frac{1}{2}$ minutes), and the blood was blown into a bead flask previously iced; after 4 minutes, when clotting began, it was swung for 1 minute. The blood was then centrifugated at 8000 revolutions for 5 minutes,

TABLE 115
COMPARATIVE TOXICITY OF SERUM FROM ONE RABBIT: CHILLED BEAD DEFIBRINATION; PORTION A WAS KEPT AT ROOM TEMPERATURE; PORTION B, AT 37 C.

Exper.	Guinea-Pig		Serum		Result
	Number	Weight	Intravenous Injection (c.c.)	Interval (hr.)	
A	1	182	3	—	2'55"
	2	178	"	$\frac{1}{4}$	3'30"
	3	201	"	$\frac{1}{2}$	Near-kill
	4	200	"	$\frac{3}{4}$	3'15"
	5	185	"	1	Very slight
	6	195	"	$1\frac{1}{4}$	Slight
	7	202	"	$1\frac{1}{2}$	Nil
B	8	173	"	$\frac{1}{4}$	2'55"
	9	181	"	$\frac{1}{2}$	Very severe
	10	181	"	$\frac{3}{4}$	Slight
	11	203	"	1	"
	12	205	"	$1\frac{1}{4}$	Nil
	13	210	"	$1\frac{1}{2}$	Very severe

The interval from start of bleeding to the first injection was 40 minutes; the other tests followed at times stated.

and the resulting serum was divided into 2 portions: one portion (A) was tested at once (No. 1), and the balance of the serum was kept in the room and retested at intervals; the other portion (B) was placed at 37 C., and likewise tested at 15 minute intervals.

The results given in Table 115 are also to be seen in Chart XV. It would appear that the toxicity in both portions dropped after a while, that at 37 C. seeming to be more marked. It should be noted, however, that with portion A, excluding No. 1 for purpose of comparison, there were 3 slight and 3 fatal or nearly fatal shocks, while with portion B there were also obtained 3 slight and 3 very severe or fatal shocks.

The difference was such as to hardly warrant the conclusion which at first suggested itself.

The results would have been more striking if a pair of tests were made at each interval with each serum. This would serve to bring out, as it has done in the work with anaphylatoxin, the error of placing undue emphasis upon the results of single tests.

Toxicity of Defibrinated Blood Obtained with Heated Beads.—According to Moldovan,¹¹ defibrinated rabbit blood though fatal to rabbits was rarely so to guinea-pigs. It is difficult to understand this statement, since in our work the guinea-pig proved to be very susceptible. Similarly, exception must be taken to his assertion that rabbit blood is without effect when injected before coagulation. Tables 96 to

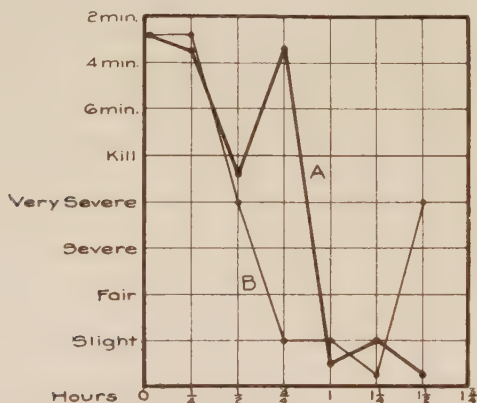


Chart 15. Comparative toxicity of normal rabbit serum: A, in room; B, at 37 C. (Table 115).

98 present sufficient evidence on this point. Of special importance is his view that the toxicity of serum and of defibrinated blood is labile at room temperature, and that exposure to 56 C. for 10 minutes results in its rapid disappearance, since large doses were without effect. It has been shown that toxicity persists at room temperature and even at 37 C.; it was in order next to determine the effect of a higher degree of heat. As is well known, anaphylatoxin is not readily destroyed at 60 C., and if the statement mentioned was correct, it would indicate that there was an essential difference between the poison of defibrinated blood and the former.

When rabbit blood is defibrinated with chilled beads, and is allowed to remain in contact with the clot and beads at room temperature, it

will be found to be acutely fatal in dose of 3 c.c., and this toxicity may persist for an hour, the duration of the experiment.

It has been pointed out that bead defibrination of rabbit blood at 40 C. seems to yield a relatively weak poison; this suggested the possibility of utilizing this method as a means of producing rabbit serum of a minimal toxicity. With this in mind, the bloods of 6 rabbits were tested. The freshly drawn blood was blown into bead flasks, previously warmed in a water-bath to 40 or 42 C., and defibrinated at this temperature, after which it was kept in the room and tested at intervals. The defibrinated blood thus obtained was found to be practically non-

TABLE 116

PRODUCTION OF TOXICITY IN RABBIT BLOOD: BEAD DEFIBRINATED AND KEPT AT 55 C. (A);
AND AT 60 C. (B, C)

Exper.	Guinea-Pig		Blood		Result
	Number	Weight	Intravenous Injection (c.c.)	Interval* (min.)	
A	1	202	3	19	Very severe
	2	205	"	34	Severe
	3	201	4	50	4'50"
	4	202	"	73	Fair
	5	192	"	84	Good
	6	198	"	99	3'
B	7	222	7	15	2'35"
	8	205	5	18	2'15"
	9	210	3	22	Very severe
	10	202	5	30	2'10"
C	11	210	5	14	3'45"
	12	205	3	21	Near-kill
	13	210	3	31	7'30"

* The interval represents the time from start of bleeding to the injection.

toxic in doses up to 7 c.c., though in one instance 5 c.c. proved fatal. As a rule, 10 c.c., representing 6 c.c. of serum, were fatal, causing either acute or more often very delayed deaths (up to 72 minutes). Even 12 c.c. could be given without effect in some animals, though usually it was fatal. A striking fact in connection with these tests was that the toxicity persisted in these defibrinated bloods throughout the duration of the experiments, $\frac{3}{4}$ to $1\frac{1}{4}$ hours.

These results led to similar experiments, in which the defibrination was effected in bead flasks, previously warmed to 45, 50, 55, and 60 C., after which the blood was kept at room temperature. Here again the

bloods were found to be acutely toxic in dose of from 5 to 10 c.c., smaller amounts not being tested. The toxicity persisted through the duration of the experiments, up to 45 minutes.

Inasmuch as the blood in all of these experiments was kept at room temperature, it was deemed important to ascertain whether the toxicity persisted at 55 or 60 C. Three experiments of this kind are given in Table 116. For these the blood was drawn into carotid pipets and at once blown into bead flasks, kept in a water-bath at 55 or 60 C.; the defibrination was effected in the bath, and the flasks were left there.

The results given in Table 116 demonstrate that the primary toxicity of rabbit blood was not destroyed in 1 hour 40 minutes at 55 C., or in half an hour at 60 C. It was not possible to make additional tests, since in both experiments at 60 C. the blood was coagulated solid in 45 minutes.

Even 3 c.c. of the blood, representing 1.8 c.c. of serum, may be fatally toxic (No. 13). It must not be inferred that the poison was made at these high temperatures. As pointed out in connection with Table 44 (see also Part IX), it is more likely that it is produced while the temperature is rising and before it passes much beyond 50 C. An exposure for a short time at 50 C. suffices to inactivate the matrix of anaphylatoxin.

THE TOXICITY OF NORMAL GUINEA-PIG BLOOD AND SERUM

In investigating the toxicity of the blood and serum of the guinea-pig, one is confronted with difficulties which are not encountered in that of the rabbit. The size of the animals is such as to preclude the obtaining of a large amount of blood from one animal. Consequently, if a series of tests is to be made, a pool of the bloods of several animals must be employed. This is objectionable, since individual variations in the blood source cannot be taken into account, and especially because one cannot measure accurately the time relations of coagulation and toxicity. The first series of experiments performed were analogous to those given in Table 96. They established the fact that guinea-pig blood, like that of the rabbit, is not toxic at the moment of withdrawal, but develops toxicity in the precoagulation period.

Toxicity of Fresh Undefibrinated Guinea-Pig Blood.—Each test given in Table 117, represents a separate transfusion. A single donor served for Nos. 1 and 2; another was used for Nos. 3 and 4; and still another for Nos. 5 to 9. For each test the blood was drawn by punc-

ture through the thoracic wall; the blood was received into a syringe previously rinsed in salt solution. It was then kept in the syringe for different intervals of time, and finally injected into guinea-pigs.

Three cubic centimeters of such blood was not sufficient to produce a serious effect, even when held in the syringe for from 3 to 3½ minutes. This dose of rabbit blood, under these conditions, gave either a severe or a fatal shock. It is noteworthy that in Nos. 3 and 4 clotting had already started in the syringe when the injections were made. This was apparent by the pressure that had to be exerted to make the injections; and yet there was but little effect produced. The injection of clot per se, with its accompanying thrombin, is therefore not the cause of the severe effects which may follow an injection of this blood.

TABLE 117
TRANSFUSION OF NORMAL GUINEA-PIG BLOOD TO GUINEA-PIGS

Recipient			Interval*	Result
Number	Weight	Blood Transferred (c.c.)		
1	205	3	1'	Nil
2	240	"	2'	"
3	184	"	3'23"	Very slight
4	195	"	4'	" "
5	185	"	3'	Fair
6	185	5	30"	Nil
7	182	"	2'	Slight
8	178	"	3'	5'50"
9	210	"	3'	8'30"

* This is the time from end of bleeding to end of injection, i. e., time in syringe. The drawing of the blood required from 10 to 30 seconds, as did also the injection.

When a dose of 5 c.c. of blood is used, the result is different. Here also, injections made at short intervals (Nos. 6 and 7) produced little effect, just as in the case of a corresponding test with rabbit blood (Table 96, No. 8). With intervals of 3 minutes, however, the 5 c.c. dose killed with typical symptoms of shock. The animal was excited, breathed rapidly, then became dyspneic, developed spasms, severe convulsions, was thrown on its side, and died with the typical agonal reflex. The autopsy findings in all of the fatal cases resulting from the injection of guinea-pig blood or serum were typical of anaphylatoxic poisoning.

It will be seen, therefore, that homologous blood in the case of the guinea-pig is distinctly less toxic than the heterologous rat or rabbit

blood. A blood may be toxic not only to the homologous animal, but even to the donor, though we have made no direct tests with the latter.

Toxicity of Normal Guinea-Pig Serum: Rod Defibrination.—For the experiment given in Table 118, 5 guinea-pigs were bled as rapidly as possible. The Novy heart pipet (Fig. 1) was employed in drawing the blood. The animals were anesthetized and bled from the exposed heart. Rod defibrination was carried out by an assistant. The total time of bleeding, from the start of the first to the end of the last bleed, was 12½ minutes. The blood from the 5 pipets was then drawn up into a bulb pipet and transferred at once to 2 large centrifuge tubes. These were whirled at 8000 revolutions for 2 minutes, after which the serum was pooled. Of this pooled, clear serum, 6 c.c. were tested at once; the remainder was placed at 38 C. and tested at intervals of 15 minutes.

TABLE 118
APPARENT VARIATION IN TOXICITY OF NORMAL GUINEA-PIG SERUM, KEPT AT 38 C.: ROD DEFIBRINATION

Guinea-Pig		Serum		
Number	Weight	Intravenous Injection (c.c.)	Time at 38 C. (hr.)	Result
1	200	6	—	Slight
2	170	"	¼	3'40." Typical shock and autopsy
3	190	"	½	Very slight
4	180	"	¾	Nil
5	170	"	1	"

The first injection was made 29 minutes after the start of the first bleeding; the other injections are reckoned from this point.

An apparent variation in the toxicity of the serum, like that in Table 101, is to be noted. This does not mean that the amount of the poison rose and fell during the time immediately preceding and following test No. 2, but rather it indicates that the animal in the fatal test was more susceptible than the average.

It will be seen, further, that the killing dose of a normal guinea-pig serum may not be much higher than that of many samples of normal rabbit serum. The smallest dose of normal rabbit serum that we have been able to kill with is 2 c.c. (Tables 199 and 110) and 1 c.c. (Table 97). Differences between the toxicity of normal rabbit and guinea-pig sera, however, do exist, since out of a given number of trials, the serum of the latter shows a much lower percentage of severe shocks and deaths than does that of the former species.

Toxicity of Normal Guinea-Pig Serum after Spontaneous Clotting.

—The previous work had shown that the toxicity of a serum was affected by the mode of defibrination, and that spontaneous clotting gave distinctly more toxic sera than such as were obtained by means of the rod. It was therefore of interest to see if guinea-pig serum would be influenced in like manner.

For Exper. A, Table 119, 5 guinea-pigs were bled in rapid succession, the blood being drawn into a heart pipet, and at once blown into a large centrifuge tube. The time from the start of the first to the end

TABLE 119

EFFECT OF SPONTANEOUS CLOTTING ON TOXICITY OF GUINEA-PIG SERUM: CONTACT WITH CLOT FOR 20 MINUTES (A); VARIABLE CONTACT (B AND C)

Exper.	Guinea-Pig		Serum			Result
	Number	Weight	Intravenous Injection (c.c.)	Time at Room Temperature	Clot Contact*	
A	1	202	3	—	20'	Slight
	2	193	6	5'	"	Very slight
	3	201	"	22'	"	23'15"
	4	182	"	35'	"	Severe
B	5	208	6	24'	5'	Nil
	6	175	"	29'	10'	7'35"
	7	199	"	35'	15'	Slight
	8	203	"	40'	20'	"
	9	210	"	47'	30'	"
	10	198	"	1 hr. 22'	1 hr.	3'15"
	11	196	"	18 hr. 45"	18 hr. 30'	3'40"
C	12	182	6	30'	10'	Good
	13	181	"	1 hr 19'	1 hr.	20'
	14	201	"	13 " 34'	18 hr. 15'	Nil
	14a	181	"	" " "	" " "	Slight

* By contact time is designated the interval from start of bleeding to beginning of centrifugation at 8000 revolutions, i. e., time of contact with clot.

The injection of No. 1 was made 1 hour after the start of bleeding; the injections of Nos. 2 to 4 are reckoned from this point, the same pooled serum being used.

Nos. 5 to 14 were each made with the serum of a single donor, and the time given represents the interval from the start of bleeding to end of injection.

of the last bleed was 6 minutes. Within 20 minutes after starting to bleed, this pooled, spontaneously coagulated blood was centrifuging at 8000 revolutions, and this was continued for 15 minutes. The resulting pooled serum was then pipetted off and tested at once in dose of 3 c.c. (No. 1); the remainder of the serum was kept at room temperature and tested at intervals shown in the table (Nos. 2 to 4).

Of 3 tests with dose of 6 c.c., one was fatal. The shock in this case resembled in a striking manner (nasal foam), the slow death

occurring with feebly toxic rabbit serum. The autopsy findings confirmed the resemblance shown in the symptoms complex; lungs in maximal distention, peppered with hemorrhages and edematous; heart beating and clot absent.

Although the time of contact of clot with the serum in Exper. A of Table 119 was but 20 minutes, the injection of No. 1 was not made till 1 hour after starting to bleed, this delay being chiefly due to the long time required for the high speed centrifuge to come to a rest. The result was not very satisfactory; consequently, a series of tests were planned, with varying clot contact, to determine the effect upon the toxicity of the serum.

Exper. B of Table 119 represents such a series of tests. For these tests, 7 guinea-pigs were bled from the heart as usual, and the blood of each animal was blown at once into separate centrifuge tubes which were then kept undisturbed for different intervals of time at room temperature. They were then centrifuged at 8000 revolutions for 5 minutes, the machine being brought to rest as soon as possible by applying the brake. Each serum was then tested with the result shown in the table.

Of the 7 tests, 3 gave acutely fatal shocks which were typical; as in Test No. 3, more or less foam flowed from the nose, and on autopsy the distended lungs were found to be peppered with hemorrhages and edematous, the heart was beating, and free of clot.

The success of these tests led to a repetition of Nos. 6, 10, and 11 on the following day (Exper. C). For this purpose, 3 guinea-pigs were bled, and the bloods were treated as before. The serum used for Nos. 14 and 14a was very lipoidal; this fact may have something to do with the result. Of the 3 sera thus tested, only 1 proved fatal; the autopsy in this case, made 14 minutes after death, showed a slight auricular heart beat, no clot, lungs distended and very edematous.

In all of these tests the greatest precautions to insure sterility were taken to avoid the possibility of the formation of a bacterial anaphylatoxin. The sera remained perfectly sterile.

The few workers who have dealt with the toxicity of normal guinea-pig serum have laid stress upon the idea that it disappears rapidly after coagulation. Slatinéano and Ciuca²⁰ tested such serum 30 minutes after bleeding and found that it produced slight symptoms of shock in guinea-pigs in dose of 5 c.c.; severe effects in 10 c.c. dose, and that it killed typically when 13 c.c. were injected. Tested 24 hours

later, the serum had become innocuous, as evidenced by a single trial; without doubt, further tests would have shown that toxicity was still present. As shown in test No. 11, a guinea-pig serum kept in contact with its clot for 18 hours is toxic in dose of 6 c.c.

In contrast to the results of the above-named investigators, it is to be noted that a lethal dose of 6 c.c. can be easily obtained. We have shown that this can be done, either by rod defibrination, or by allowing the serum to remain for some time in contact with the clot. Particularly noteworthy is the fact of variation in toxicity of the sera of different animals. Thus, in Expts. B and C where each test represents a different serum, it will be seen that with like dosage only 4 of 10 sera were toxic. This difference in results, however, may be due in part to the variable resistance of the test animals, since evidence of such variation has been presented heretofore.

Toxicity of Normal Guinea-Pig Serum: Bead Defibrination.—In view of the effect of beads on the toxicity of rabbit serum, it was desirable to see if like results could be obtained with guinea-pig serum. The coagulation time of rabbit serum is much easier to control than that of the guinea-pig; the mere icing of the flask containing beads is not enough to retard noticeably the clotting of the latter blood. By packing the bead flask in a freezing mixture at -4 to -9°C ., coagulation can be somewhat retarded.

For the few attempts made by this method, the guinea-pig was bled in the usual way and the blood was blown at once into a bead flask, previously packed in a freezing mixture of salt and cracked ice at -9°C . The flask was then kept at room temperature; in 2 minutes the blood had congealed, but on slight shaking it became liquid. In another 2 minutes, when coagulation did set in, the flask was shaken for 30 seconds; the blood was then decanted into a centrifuge tube and whirled at 3000 revolutions for 5 minutes. The resulting serum was tested on 2 guinea-pigs. No. 1 (187 gm.) received 2 c.c., 18 minutes after the start of bleed; at once severe dyspnea and spasms occurred. In No. 2 (205 gm.) 2.5 c.c. gave a near-kill: severe dyspnea, convulsions, thrown in 1 minute, complete suppression of respiration, but eventual recovery.

These experiments were not carried further, but they indicate that with appropriate methods one can obtain a normal serum, which, even in small dose, is by no means harmless for the homologous animal. It is possible that 3 c.c. of the serum used in the preceding experiments would have proven fatal.

The observation of Wassermann and Keysser³⁶ that pooled normal guinea-pig serum could be toxic in 4, 3 and even 2 c.c. is deserving of mention at this point. Assuming that due care was taken to exclude anaphylatoxin-production by bacterial contamination, and that the recipients were not abnormally weak, there remain 2 possible causes for this unusual toxicity.

It has been shown that normal rabbits may possess a blood which is fatal on transfusion in dose of 2 and even 1 c.c., and that the serum from such animal may be also toxic in dose of 1 c.c. (Table 97). From such observations it might be assumed that normal guinea-pigs will show a like variation. Without doubt, the blood of every species of animal is subject to changes of this kind but the limits of such variation are different and depend upon the susceptibility of the animal. Guinea-pigs are very susceptible, but they are not all equally so. This is seen in the fact that with a given dose of poison half of the animals in a series may die while the other half show no effect. But it is reasonable to believe that with 2 or 3 multiples of such dose all of the test animals will die. It follows, in other words, that the guinea-pig, unlike the rabbit, cannot be a carrier of more than 1 or 2 average lethal doses of poison per 200 gm. of body-weight. On this basis, it might be possible to obtain a guinea-pig serum which would be initially toxic in 5 c.c. dose, but it would be difficult to account thus for the 2 c.c. dose. Hence a variable inherent toxicity of the blood cannot account for the highly poisonous, pooled serum. Rather the cause must be looked for in the method of getting the blood, especially in coagulation conditions. The latter being favorable, it is possible to obtain a serum which will be toxic in 3 c.c. dose.

Toxicity of Guinea-Pig Blood: Rod Defibrination.—Some experiments to show the comparative effects of rod and bead defibrination on the toxicity of guinea-pig blood were also carried out. The blood was used in preference to serum because it was possible to make the injections much sooner after bleeding, the time consumed in centrifugation being eliminated.

A number of tests were made with blood defibrinated with the glass rod; of these, 3 are given in series A of Table 120. They serve to show that such whipped blood is not very toxic, since 5 c.c. can be given without much effect. As shown in Table 99, No. 2, 3 c.c. of defibrinated rabbit blood may be acutely fatal.

³⁶ Folia serol., 1911, 7, p. 243; Ztschr. f. Hyg. u. Infektionskr., 1911, 68, pp. 541, 544; Centrabl. f. Bakterirol., I, Ref., 1911, 50, Beiheft, pp. 52, 72, 78.

For the tests mentioned, 3 guinea-pigs were bled into heart pipets and each blood was at once whipped with the rod. The bloods were defibrinated for 5, 3, and 2 minutes, respectively, and tested in the order given on guinea-pigs Nos. 1, 2, and 3. The injections were made as rapidly as possible after the completion of the whipping of each blood, yet notwithstanding this fact, the bloods proved to be rather harmless.

Toxicity of Guinea-Pig Blood: Chilled Bead Defibrination.—In view of these unpromising results, bead defibrination was next resorted to, and rather striking results were obtained, as will be seen from

TABLE 120

PRODUCTION OF TOXICITY IN GUINEA-PIG BLOOD: ROD DEFIBRINATED (A); CHILLED BEADS (B)

Series	Exper.	Guinea-Pig		Blood		Result
		Number	Weight	Intravenous Injection (c.c.)	Interval*	
A	1	1	184	5	6'	Slight
		2	207	"	3'45"	Fair
		3	191	"	3'	Very slight
B	2	4	193	3	14'	46'
		5	201	4	17'	Fair
	3	6	178	3	10'	23'
		7	175	4	17'	30'
	4	8	177	3	11'	Very slight
		9	212	5	26'	Fair
	5	10	181	6	6'	2'20"
		11	179	3	14'	3'30"
	6	12	210	3	10'	Near-kill
		13	200	4	18'	6'10"
		14	190	3 (serum)	20'	Very slight
	7	15	198	3	12'	Nil
		16	205	4	23'	Very severe
		17	180	3 (serum)	16'	" slight

* The interval (Nos. 1 to 3) indicates the time from end of bleeding to end of injection; for the others, it is time from the start of bleeding to end of injection.

Series B of Table 120. They are not as marked, however, as those with rabbit blood. Furthermore, it must be emphasized that the fatal effects obtained in these experiments with small doses of defibrinated blood, are not necessarily due to the contained serum. Thus, the death following the injection of 3 c.c. of the blood does not mean that 1.5 c.c. of the serum would be fatal. This is clear from the results of Experiments 6 and 7, where tests were made with the blood and also with the serum.

For Expers. 2, 3, and 4 the bead flasks were previously chilled by being placed in a freezing mixture of salt and cracked ice at about -4°C . Large guinea-pigs were bled into heart pipets, and the blood of each was at once blown into a corresponding chilled bead flask, which was then kept at room temperature. In Exper. 2 the blood began to show a surface coagulation in 6 minutes; it was then swung gently for 2 minutes, but this was not sufficient, since a secondary clot formed; hence it was again gently agitated for half a minute. The retarding effect of the cold beads, however, was such that a third clotting developed, but this was disposed of by swinging for 10 seconds. This blood in dose of 3 c.c. caused a delayed death (No. 4).

In Exper. 3 the coagulation also began in 6 minutes; after agitation for 2 minutes, the blood was at once injected into Nos. 6 and 7, with fatal results.

In Exper. 4 a firm clot developed in the flask in 2 minutes. This was vigorously shaken for 2 minutes, but the fibrin failed to collect on the beads; this necessitated filtering the blood through cotton. The results were mild, probably due to the rapid coagulation of the blood.

For Exper. 5 the bead flask was chilled outside the window at about -10°C . for 10 minutes. After addition of the guinea-pig blood, the contents were given at once a gentle swing for 4 minutes at room temperature. A portion was then tested and, inasmuch as it gave a very acute death, which might be interpreted as due to partially unclotted blood, the remainder was filtered through cotton and injected into No. 11. This likewise caused a typical acute fatal shock. The autopsies were made 9 and 4 minutes, respectively, after death and were typical: maximal distention of lungs, heart beating, no clot. Three c.c. of such defibrinated blood, representing 1.8 c.c. of serum or less, should be fatal for the homologous animal. The toxic effects, however, must not be ascribed wholly to the serum present, but rather to a catalyzing substance which induces further poison production in the recipient, as already pointed out.

Another experiment similar to No. 5 was performed at the same time. The bead flask was cooled as before, and after adding the blood, it was at once gently swung for 6 minutes at room temperature, after which it was set aside for another 6 minutes. This blood, tested 13 minutes after end of bleed in dose of 3 c.c., caused death in about 4 hours; 4 c.c. produced a good shock, but the animal recovered.

In still another experiment, in which the bead flask was not chilled, after defibrination for 3 minutes at room temperature, a test with

2.5 c.c. was made 6 minutes after start of bleed and caused typical shock and death in 5'30"; the autopsy performed 10 minutes after death was likewise typical: the heart still beating, absence of clot, and maximal distention of lungs. The same blood left in contact with the beads was retested 12 minutes later, and in 3 c.c. dose it caused death in 3 hours.

Of five other bloods, tested in like manner, unchilled beads being used, the defibrination ranging from 2 to 6 minutes, only one caused deaths which were delayed for about 3 hours.

In 2 other experiments, which were made at the same time as Exper. 5, but with unchilled beads, the defibrination being 3 minutes, 5 c.c. of one and 6 c.c. of the other injected 4 and 8 minutes, respectively, after the end of bleeding, gave only moderate shock effects.

For Exper. 6 an old female in advanced pregnancy was employed. The blood was received into a chilled flask and kept in the room for 3 minutes when it was gently swung for 1 minute. It will be noted that 3 c.c. gave a near-kill, while 4 c.c., caused death in 6'10". The serum from this blood, however, in 3 c.c. dose produced only a very slight effect. In Exper. 7 the blood and serum of a nonpregnant female were tested in the same way, and while 4 c.c. of the former produced a very severe shock, the serum was again without much effect. A similar result as regards the toxicity of blood and serum was noted in another experiment with blood from an animal in advanced pregnancy. These results show that the poisonous effect of defibrinated blood is not wholly due to the soluble poison contained in the serum, but that an additional factor is involved.

These various results are mentioned for the purpose of bringing out the fact that the individual bloods appear to vary in their poison production during coagulation, just as guinea-pig serum of different origin varies in the ease and extent to which it gives rise to anaphylatoxin, when treated with agar or other inducing agents. Incidentally, they show that defibrination with unchilled beads does not give as high a percentage of toxic bloods as is obtained when thoroughly chilled beads are employed.

THE TOXICITY OF NORMAL RAT SERUM

This serum, because of the extreme ease with which it can be toxified by alien substances, is of special interest. The influence of the different methods of defibrination on the toxicity has not been investigated. We have observed, however, that mere incubation of sterile rat

serum for a long time, 12 to 24 or more hours, often renders it toxic, so that 1 c.c. is fatal to a guinea-pig of 200 gm. Fresh normal rat serum, when obtained by the ordinary method of rod defibrination, is rarely harmful in dose of 5 to 6 c.c. It is needful, then, only to incubate such serum, at about 37 C., to disturb in some manner its equilibrium and thereby bring about poison production. While the sera of rabbits and guinea-pigs can be made quite toxic by certain methods of defibrination, we have never noticed that mere incubation exerts so great an action upon their toxicity.

In toxicity tests with rat serum, as with all other sera, it is necessary to remember that two variable factors are involved in the experiment. One lies in the individuality of the donor which may be seen in the ease or difficulty with which its serum may be toxified; the other

TABLE 121
APPARENT VARIATION IN THE TOXICITY OF RAT SERUM KEPT AT 37 C.

Guinea-Pig		Serum		
Number	Weight	Intravenous Injection (c.c.)	Time at 37 C. (hr.)	Result
1	195	4	—	Nil
2	185	"	1/4	4'40"
3	179	"	1/2	Slight
4	190	"	3/4	Moderate
5	201	"	1	4'5"
6	204	"	1 1/4	Fair
7	166	"	1 1/2	Slight
8	193	"	1 3/4	3'40"

The first injection was made 1 1/4 hr. after the start of the first bleeding.

appears in a like peculiarity of the recipient and is recognized as a variable resistance on the part of the animal to the poison. This latter factor is well brought out in Table 121.

For the experiment there given, 10 normal rats were bled into heart pipets. Each blood was defibrinated separately by means of the rod. The blood was then pooled and centrifuged at 3000 revolutions for one hour. A portion of the serum thus obtained was tested at once (No. 1); the balance was placed at 37 C. and tested at intervals of 15 minutes. The results of these tests are shown in the table and in the Chart 16. It will be seen that of the 8 tests, 3 resulted in acute death. The symptoms were typical of anaphylactic shock, as also were the autopsy findings, the examinations being made 3 minutes after death.

It might be supposed that the poison fluctuated in amount, but it has been firmly established that such is not the case. The observed variation must be ascribed to the varying resistance of the test animals. In this experiment the short duration of incubation probably has no effect upon the toxicity; it follows then that the poison was present in the pooled serum as soon as prepared. Once developed, it persists for a long period of time, the same as in any anaphylatoxic serum.

Lastly, as in the case of other sera, the toxicity of rat serum is influenced by clot contact. In connection with another experiment (page 533), a pooled serum was prepared and tested. The blood from each animal was blown from the heart pipet into a common cylinder. The blood of 8 rats was thus collected in half an hour. The coagulated

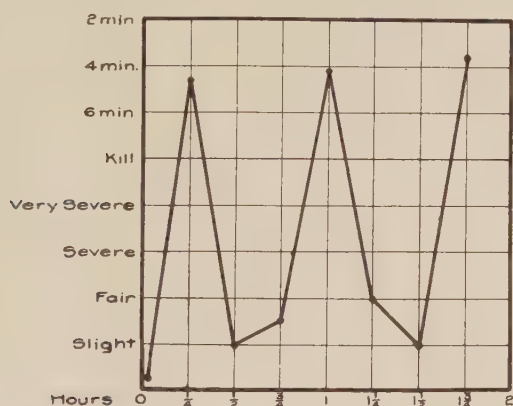


Chart 16. Apparent variation in toxicity of normal rat serum (Table 121).

mass was then defibrinated with a glass rod, and the blood centrifuged at 3000 revolutions. The serum was set aside at 0 C. for 5½ hours before it was tested; consequently, the injections were made about 6¼ hours after start of bleeding.

A guinea-pig of 170 gm. which received 4 c.c. of this serum responded with a typical shock and death in 4'30". Another of 185 gm. was given 3 c.c. with the same result, death occurring in 3'30". The injection of 2 c.c. gave but a slight effect. It will be seen, therefore, that the toxicity of normal rat serum can be easily doubled by mere clot contact. In view of the great lability of rat serum, it should be possible to toxify it by such contact to the same extent as in case of rabbit serum.

SUMMARY

Very rapid transfusion of the blood of normal rabbit to the guinea-pig or white rat shows that the toxicity of such blood is subject to great variation. It is often possible to transfuse 10 c.c. of heart blood without much effect. Exceptionally, however, the blood from apparently normal rabbits is inherently toxic in dose of 2 or even 1 c.c., and the plasma and serum from such blood may be correspondingly toxic (Table 97).

The natural resistance of the rabbit enables it to act as a carrier of the poison which may be generated in the normal animal as a result of changed conditions, notably diet. The toxicity of the blood of an apparently normal rabbit may correspond to 100 guinea-pig lethal doses per kilo.

A blood which is initially nontoxic becomes poisonous in 3 c.c. dose, just prior to the appearance of coagulation. The speed of poison-production corresponds to that of anaphylatoxin by agar, inulin, trypanosomes, etc.

The defibrinated rabbit blood may be toxic in dose of 2 to 3 c.c., the toxicity of the preclot stage being maintained for some time.

This precoagulation toxicity, as well as that of defibrinated blood, and the inherent toxicity of normal rabbit blood may be due in part to the presence of preformed anaphylatoxin; it is probably due, chiefly, to the formation of something which acts as an accelerator or catalyzer within the recipient, thereby producing poison, *in vivo*.

This is indicated by the fact that the serum obtained from such bloods is less toxic than the poisonous blood from which it is obtained; also, by the fact that the transfusion of blood from rats injected with toxic blood may be fatal to guinea-pigs, whereas like transfusions after injection of toxic serum are negative.

Further, the rat, like the guinea-pig, is subject to acute fatal shock when injected with any one of the above mentioned 3 types of toxic bloods. In view of the great insusceptibility of the rat to anaphylatoxin, it follows that the cause of death is not the anaphylatoxin which may have been present in the injected blood; it shows that another factor is involved, an inducing agent or accelerator which acts like agar, peptone, etc.

The presence of an accelerator in preclot blood is demonstrable by injection into white rats; their blood on being transfused to guinea-pigs may cause death (Table 100). As in similar experiments with agar and peptone, a period of incubation is necessary to develop toxicity in

the recipient of the rabbit blood. Similar injections of rats with large amounts of highly toxic rabbit serum do not give evidence, on subsequent transfusion, of *in vivo* production of poison.

It is to be inferred that changes in the blood, in the preclot stage, give rise to a catalyzing agent which acts in the same way as alien substances (agar, etc.), and causes the production, *in vitro*, of some anaphylatoxin; the latter persists in the clear serum and is responsible for its primary toxicity.

Poison production and fibrin formation are parallel phenomena induced by the presence of accelerating or catalyzing substances. In each, the matrix is to be considered as undergoing an intramolecular change resulting in tautomeric modifications.

The serum is not as active as the original poisonous blood. This seeming decrease in toxicity is moderate when the serum is obtained by centrifugation at 3000 revolutions; it appears to be more marked when prepared by swinging at 8000 revolutions (Table 98).

The decrease reaches its lowest point in about 45 minutes, after which the toxicity persists unchanged for a long time (22 to 27 hours, Table 106); it is not affected by keeping at 37 C. (Table 115). This permanent toxicity of a serum is due to the soluble anaphylatoxin, while that which quickly disappears is probably due to some residual accelerator.

The decrease is not in evidence in defibrinated blood. The defibrinated blood obtained with beads, at 40 C., is relatively less toxic than such as is obtained at room temperature. On the other hand, blood which is defibrinated with beads, at 55 C., is toxic in 4 c.c. dose, and remains so; similarly, defibrinated at 60 C., the toxicity persists for the duration of the experiment (Table 116). The poison is not destroyed at such temperatures.

The toxicity of rabbit serum is influenced greatly by the conditions under which defibrination is affected. Rod defibrination gives the least toxic serum, one which kills in 6 c.c. dose. Spontaneous coagulation, with short or long contact with clot, appears to yield less toxicity than with contact of 20 minutes, the lethal dose of the latter being 2 to 3 c.c. (Table 106).

The most active serum is obtained by bead defibrination, but the temperature by influencing the speed of coagulation is of moment. Bead defibrination at 40 C. gives less toxicity than when carried out at room temperature (Table 108), or at 0 C. (Table 112). The chilling

of beads and flask, thereby retarding the coagulation, gives the best result: 2 to 3 c.c. lethal dose (Table 109). The chilled rod method appears to give no better result than the unchilled. The addition of blood which is on the point of coagulation to the chilled bead flask does not yield a very toxic serum (Table 111). Contact of serum with clot gives maximal toxicity (Table 113).

The apparent variation in the toxicity of normal rabbit, rat, or guinea-pig serum is not due to a change in the poison, but to the varying resistance of the guinea-pigs (Tables 101, 118, 121). The toxicity of the sera, apart from methods employed in defibrination, vary with the individual animal.

The symptoms and autopsy findings in guinea-pigs, after injection of toxic blood or serum, are essentially those produced by anaphylatoxin, and in anaphylactic shock. Identical results are obtained when rat or guinea-pig blood or serum is used instead of that of the rabbit. The homologous blood is distinctly less toxic than heterologous.

Large doses of normal rabbit serum which kill guinea-pigs rapidly cause death with thrombi in the heart and large vessels. This may occur with serum which is more than 24 hours old. Smaller doses kill, but at autopsy no thrombi are to be found (Tables 103, 104). The clot observed at autopsy is probably always of postmortem origin and cannot, therefore, be considered as the cause of death.

Strikingly different pictures are to be found at autopsy of animals dying from large doses of serum, depending upon whether death occurs rapidly (2 to 4 minutes), or more slowly (5 minutes to 2 hours). The findings in guinea-pigs succumbing subacutely to homologous serum resemble closely those of a slow killing rabbit serum; with acute ending they correspond to those of typical anaphylactic shock.

Sodium oxalate, when added to rabbit serum, and allowed to act for a short time, will prevent thrombosis, but will not protect against a fatal issue; a longer contact, however, may render the serum non-toxic. Similarly, the addition of sodium oxalate to preclot blood will prevent not only coagulation, but also poison-production. The recalcification of an oxalate plasma results in development of toxicity as well as coagulation (Table 105).

Guinea-pig blood, like that of the rabbit and rat, is not toxic at the moment of withdrawal, but becomes so in the preclot stage. Under like conditions, guinea-pig blood is not as toxic to a guinea-pig as is rabbit blood (Table 117). When partially clotted, it may be injected in 3 c.c. dose without effect.

On rod defibrination, guinea-pig blood may yield a serum which is toxic in dose of 6 c.c.; a like result is obtained in spontaneous clotting (Table 119). With chilled beads, it is possible to get a serum which will kill in 3 c.c. dose.

The defibrinated blood of guinea-pig, prepared with rod, is less toxic than that obtained with chilled beads; the latter may be toxic in 3 c.c. dose. As in case of rabbit blood, this toxicity is not entirely due to the poison in solution, since the serum from such blood is less toxic than its source (Table 120).

Normal rat blood may become toxic by prolonged incubation. While the serum obtained from a single rat, by rod defibrination is rarely fatal in 6 c.c. dose, it is possible, by clot contact, to increase the toxicity so that 3 c.c. will kill.

The speed of poison production in blood in the preclot stage, the effects in animals, the resistance of rats to the toxic serum, the persistence of the toxicity at room temperature, at 37 C., and especially at 55 to 60 C., indicate that anaphylatoxin is developed in normal bloods before and during coagulation. The normal toxicity of serum is, therefore, to be correlated with anaphylatoxin; the latter is produced by changes incidental to clotting; it is increased in amount when the serum is subsequently brought into contact with alien substances, such as trypanosomes, bacteria, agar, inulin, etc.

